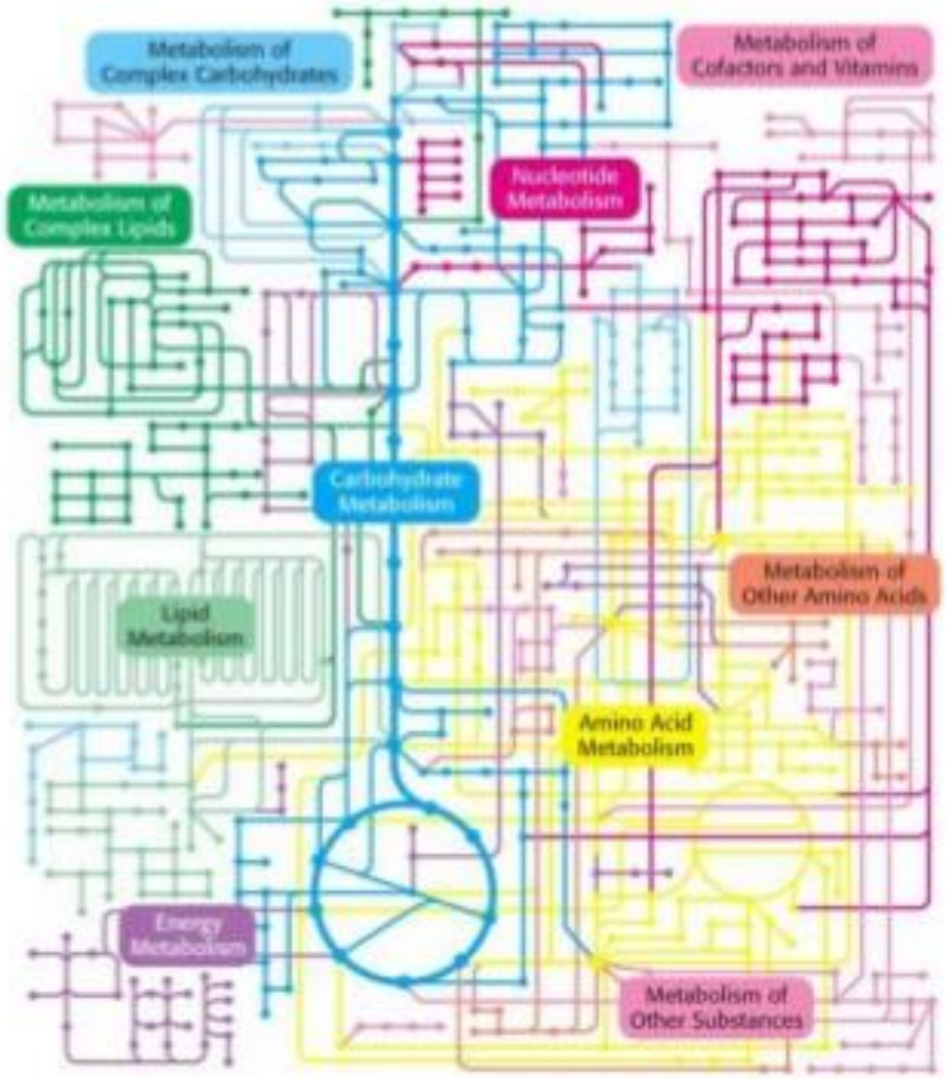
Integration of Metabolism



1. **Interconnection of pathways**
2. **Metabolic profile of organs**
3. **Food intake, starvation and obesity**
4. **Fuel choice during exercise**
5. **Ethanol alters energy metabolism**
6. **Hormonal regulation of metabolism**

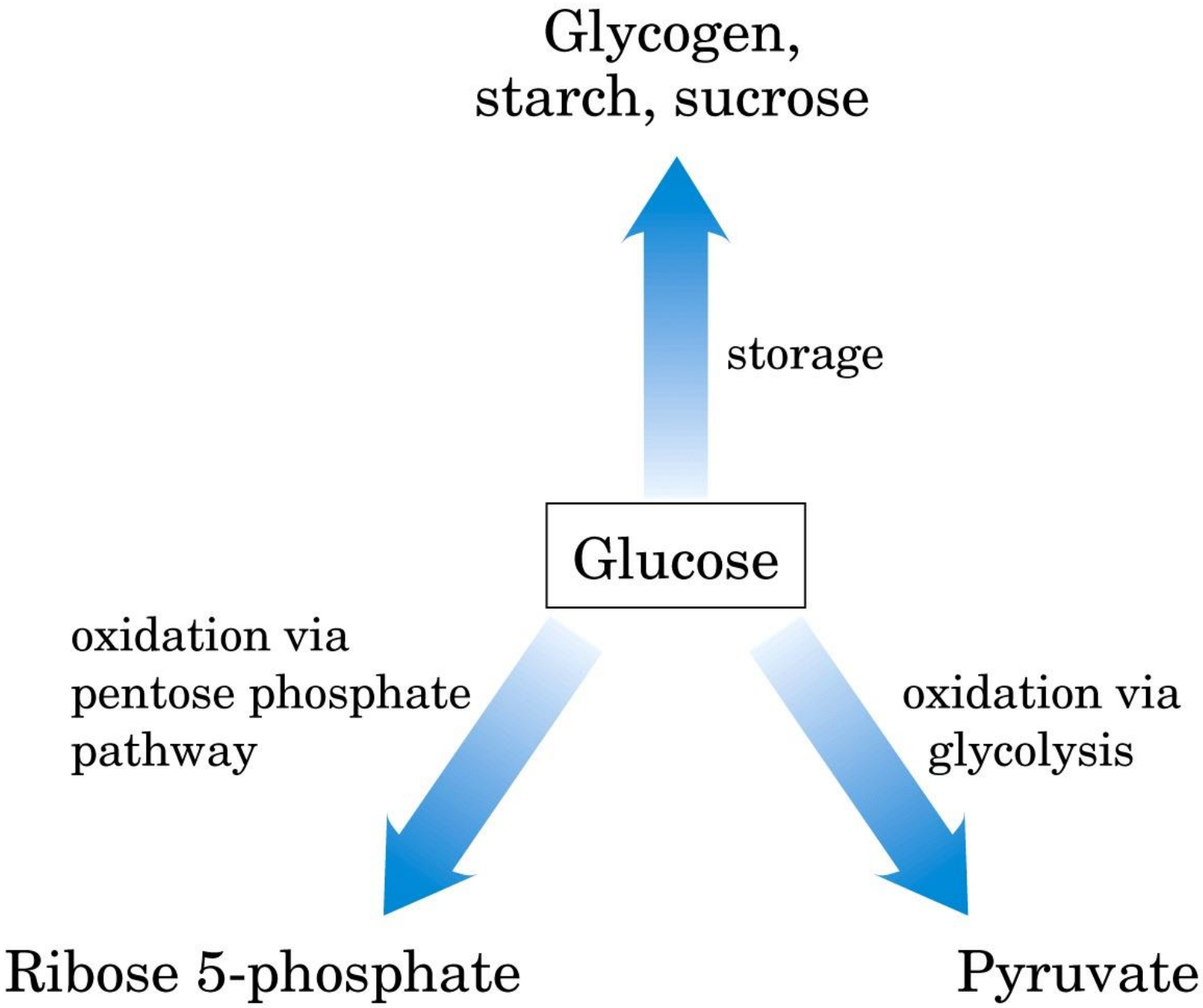
Metabolism of Carbohydrates

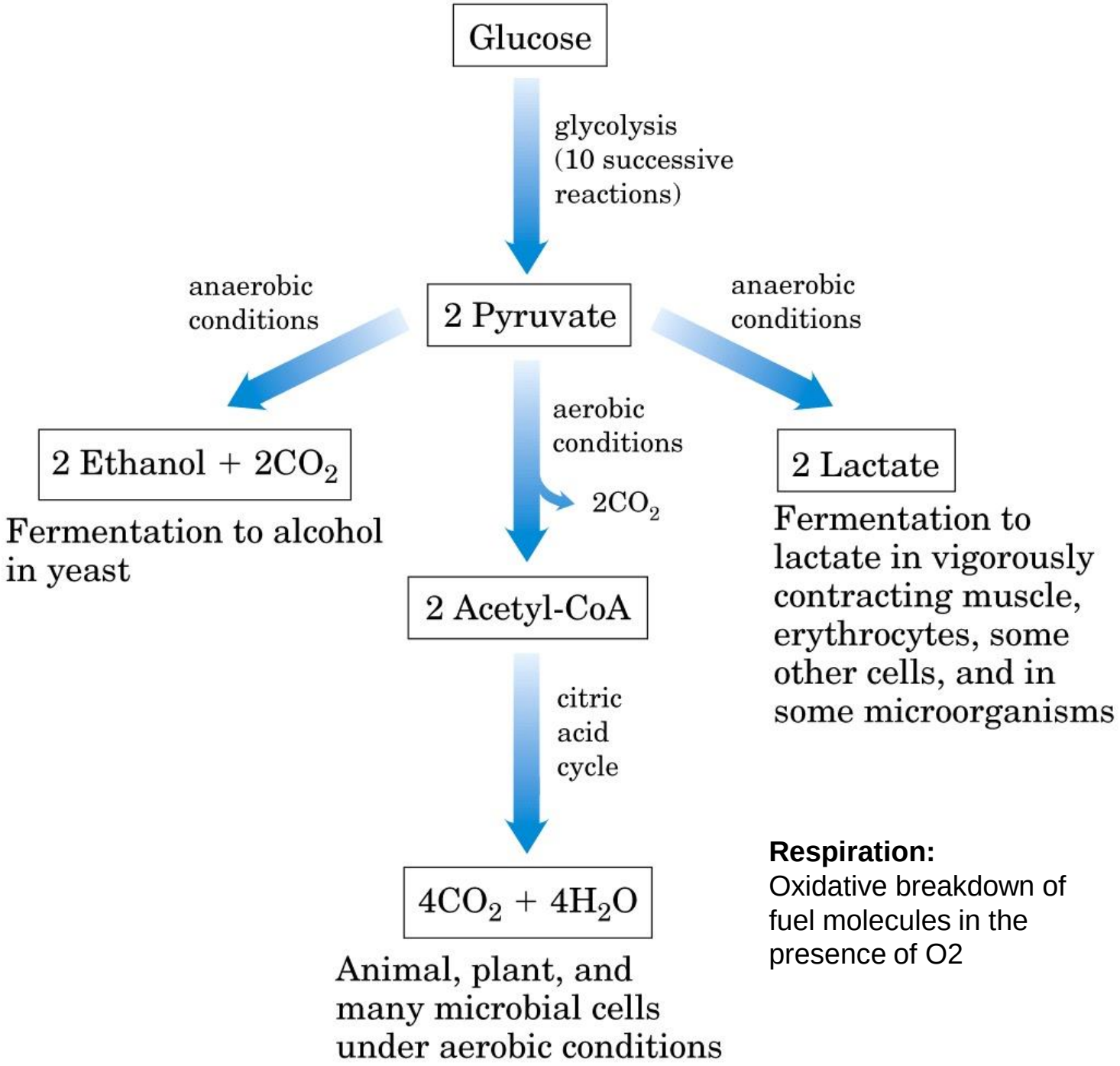
Glycolysis

A nearly universal pathway in biological systems to obtain energy from the oxidative degradation of glucose and other carbohydrates. Polysaccahrides (starch, glycogen) Disaccharides (maltose, sucrose, lactose) Monosaccharides (glucose, fructose,galactose).

Anaerobes : live in the absence of Oxygen
Aerobes: need Oxygen to oxidize their organic nutrients.
Facultative aerobes: live either aerobically or anaerobically.
Glucose is oxidized to pyruvate, which under anaerobic conditions may then be converted to lactate.

The ten enzymes of glycolysis convert glucose into 2 pyruvate
1st five reactions= Energy investment= Preparatory stage
2nd five reactions= Energy released= Payoff stage


Glycogen,
starch, sucrose
storage
Glucose
oxidation via
pentose phosphate
pathway
oxidation via
glycolysis
Ribose 5-phosphate
Pyruvate



Respiration:
Oxidative breakdown of fuel molecules in the presence of O2

Under anaerobic conditions: glycolysis consists of **11** coupled reactions with the overall net reaction being:

D-glucose + 2 Pi + 2 ADP $\longrightarrow$ 2 lactate + 2 H^+ + 2 ATP + 2 H_2O

Under aerobic conditions: glycolysis consists of **10** coupled reactions with the overall net reaction being:

D-glucose + 2 Pi + 2 ADP + 2 NAD^+ $\longrightarrow$

2 pyruvate + 2 H^+ + 2 ATP + 2 NADH + 2 H_2O

Glycolysis can be divided into 2 stages:

Stage 1 (The Preparatory Phase): Consists of the collection of sugars by the liver. (sugars other than glucose feed into glycolysis).

Subsequent phosphorylation of these sugars at the expense of 2 ATP's.

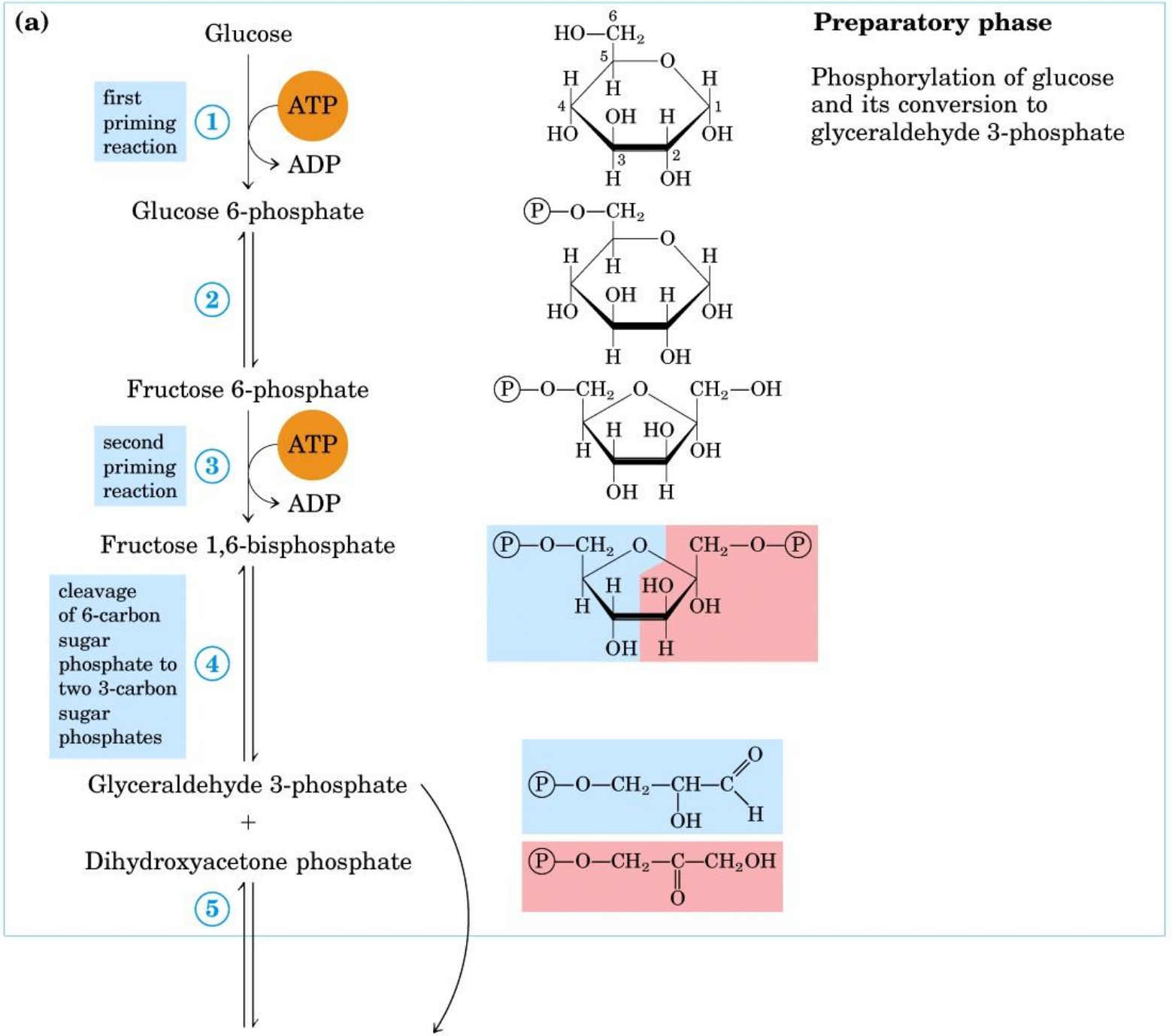
Conversion to glyceraldehyde 3-phosphate.

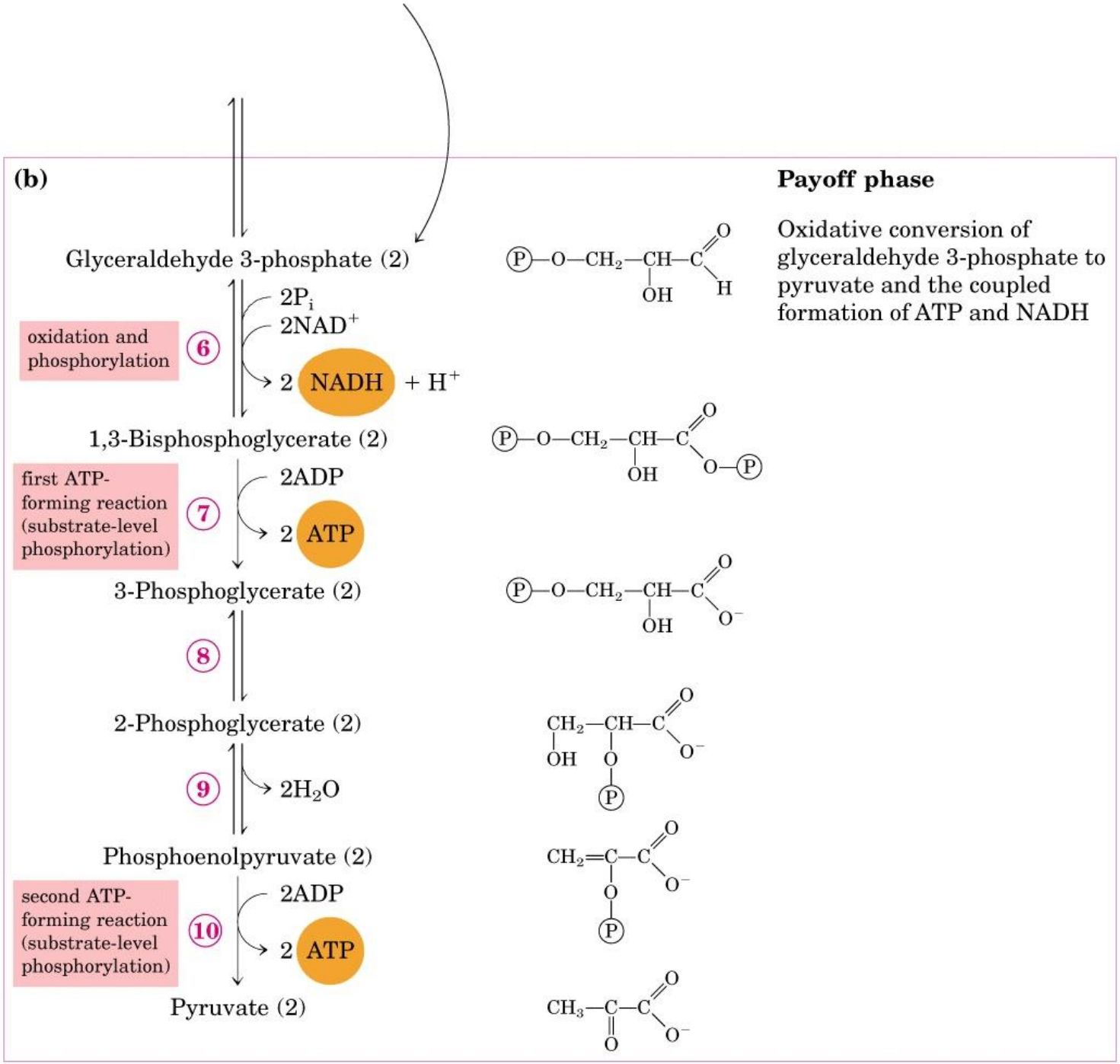
Breaks 6C into 2* 3C

Stage 2 (The Payoff Phase): Consists of conversion of glyceraldehyde 3-phosphate to either pyruvate or lactate via a series of oxidation-reduction steps.

Concomitant conservation of energy via formation of 4 ATPs

2 * 3C oxidised to 2 * pyruvate

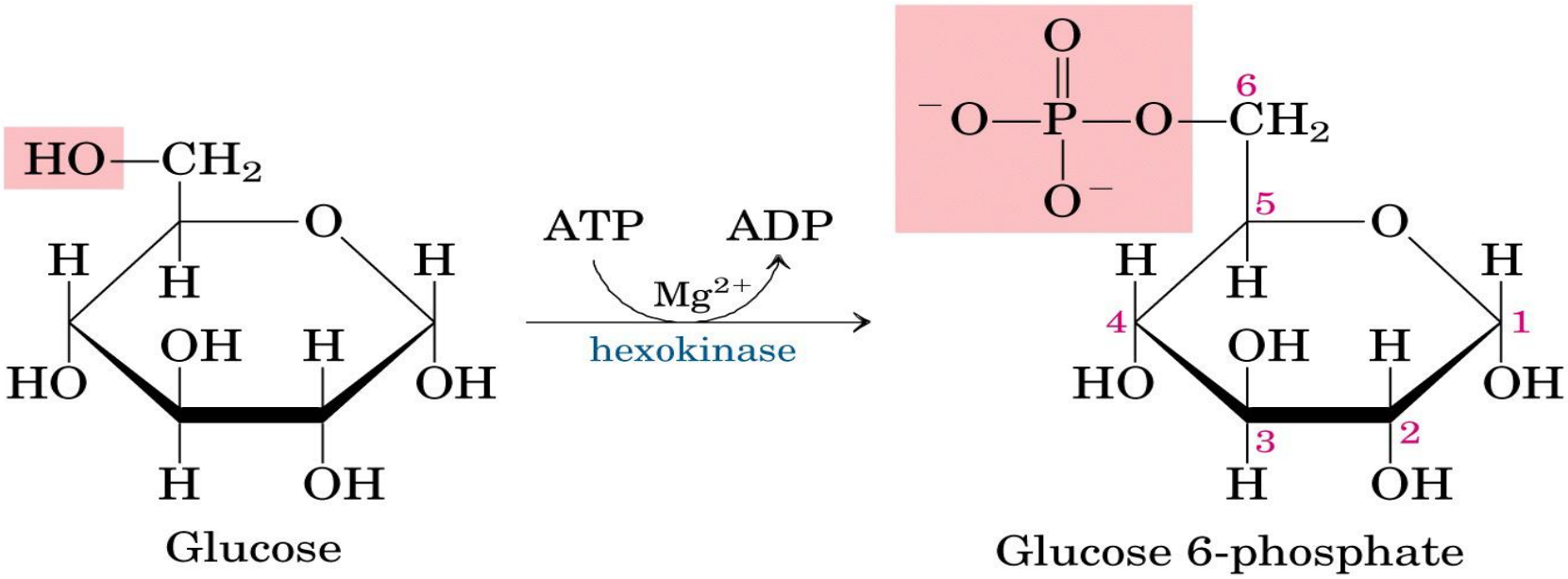

(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate


(b)
Glyceraldehyde 3-phosphate (2)
2P_i
2NAD^+
oxidation and phosphorylation
6
2 NADH + H^+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H_2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH

Glycolysis: Stage 1

Initial Strategy: **Trap glucose in the cell and convert it to a compound that can be cleaved into phosphorylated 3-carbon units. Glucose transporter will not transfer G6P.**

1st Reaction: **Glucose enters cell and is phosphorylated to glucose 6-phosphate, a negatively charged molecule which is trapped inside the cell**

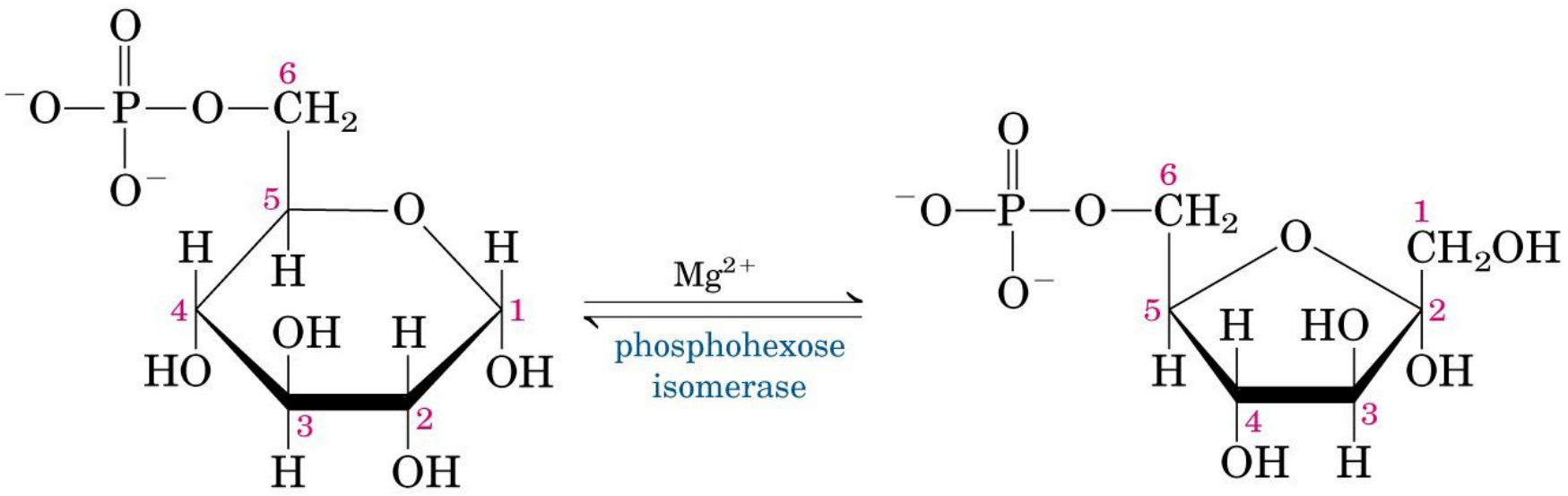


2nd Reaction: isomerization of glucose 6-phosphate to fructose 6-phosphate.

Isomerization (chemistry): transfer of a molecule to its isomer (same molecular formula but different structural formula). (Biochemistry) ketose-aldose isomerization.

6 membered pyranose ring

5 membered furanose ring



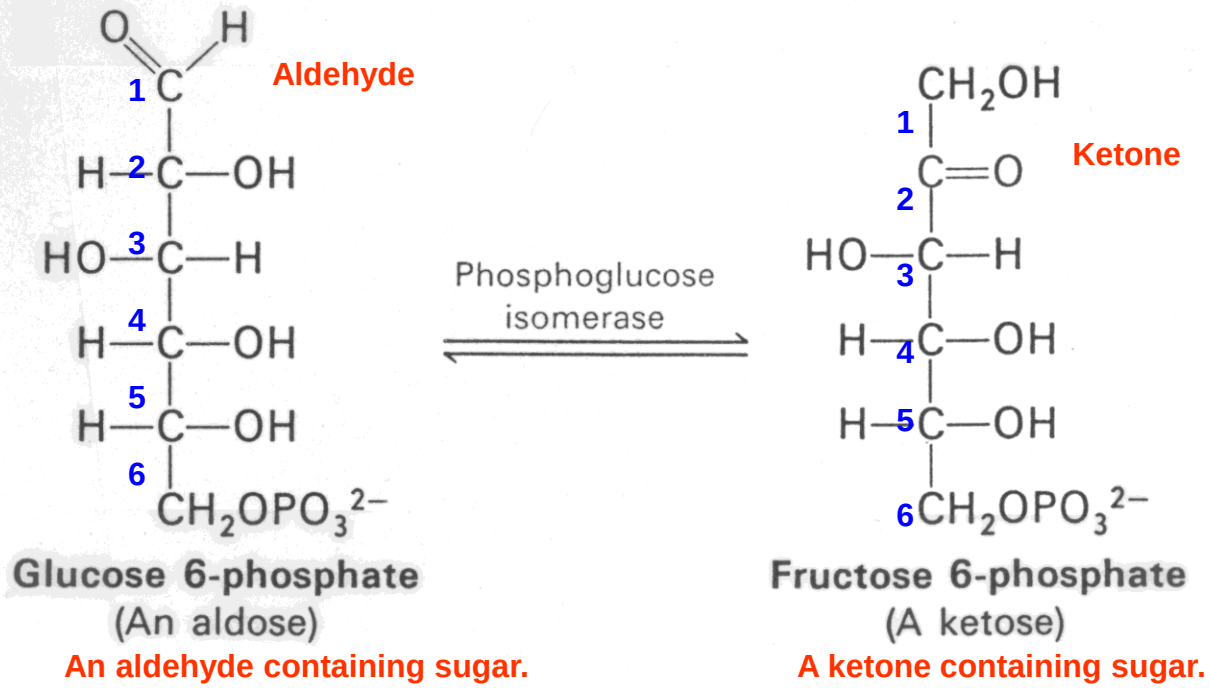
Glucose 6-phosphate

Fructose 6-phosphate

This reaction is readily reversible.

This reaction represents an example of a conversion of an *aldose* to a *ketose.*

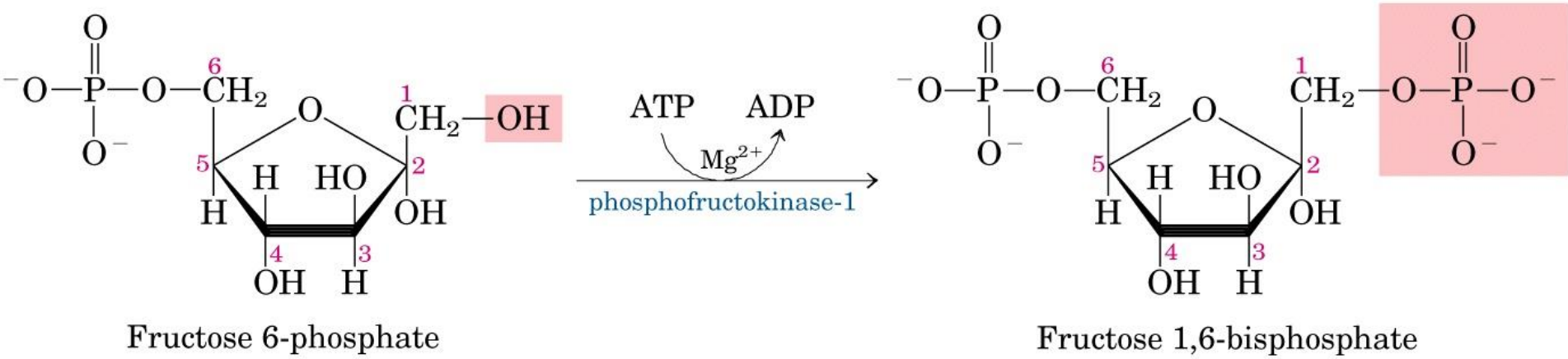
Open chain representation of the sugars.



<u>3rd Reaction:</u> Fructose 6-phosphate is phosphorylated by ATP to form fructose 1,6-bisphosphate.

This is the second of the two priming reactions in glycolysis.

Catalyzed by *phosphofructokinase (PFK; <u>PFK1</u>).*



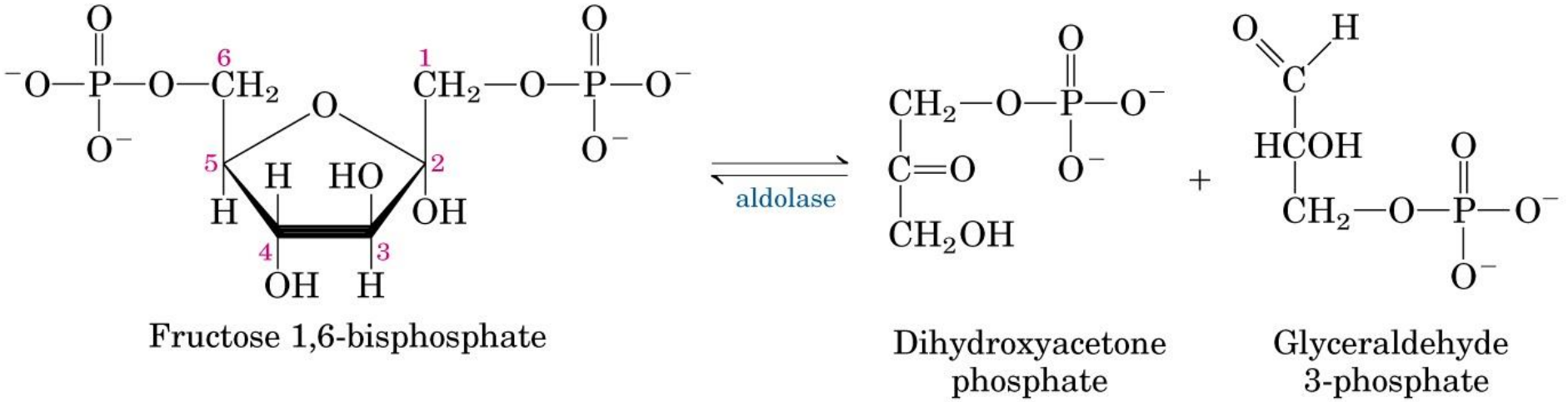
Fructose 6-phosphate

Fructose 1,6-bisphosphate

PFK is the major point of regulation in glycolysis. Rx is irreversible.

4th Reaction: Cleavage of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

Represents cleavage of a hexose into two trioses.



Fructose 1,6-bisphosphate

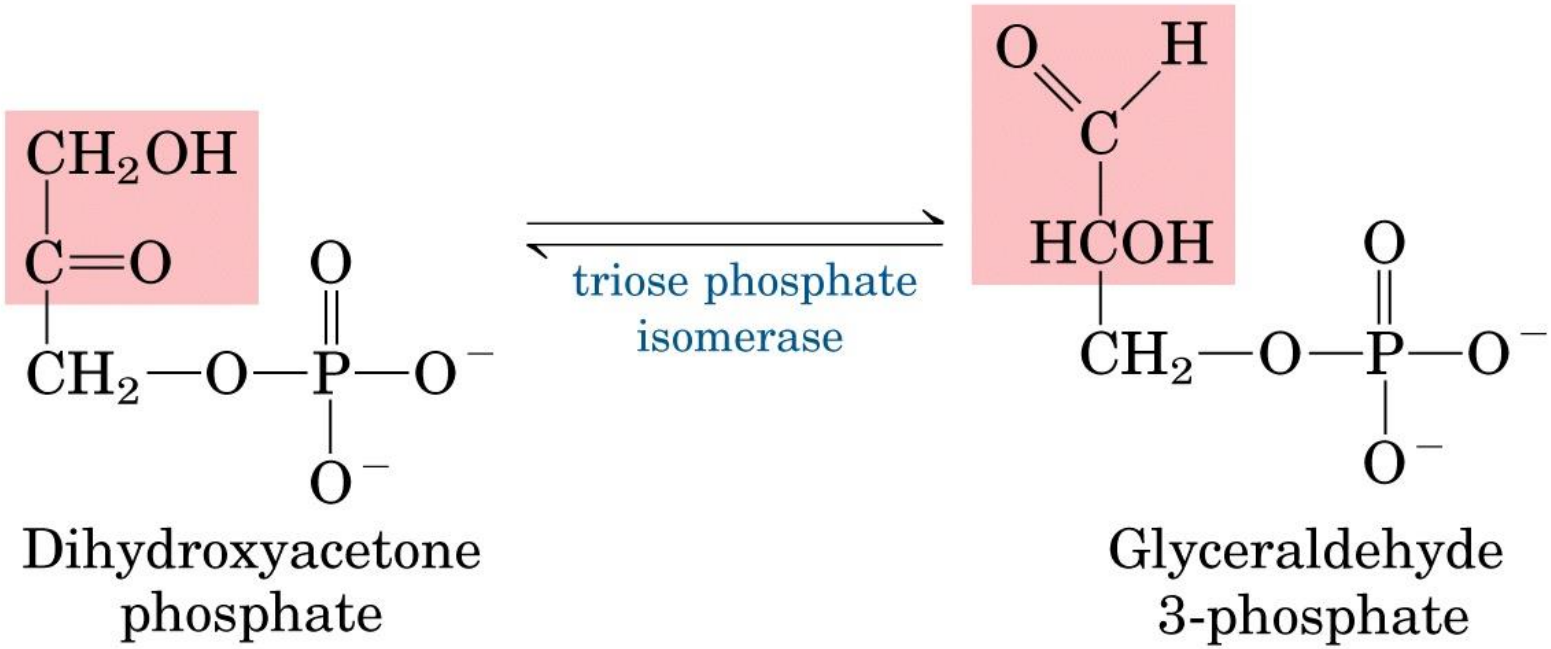
Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

Note: Reaction is readily reversible. It is pulled to the right via removal of glyceraldehyde 3-phosphate via subsequent steps.

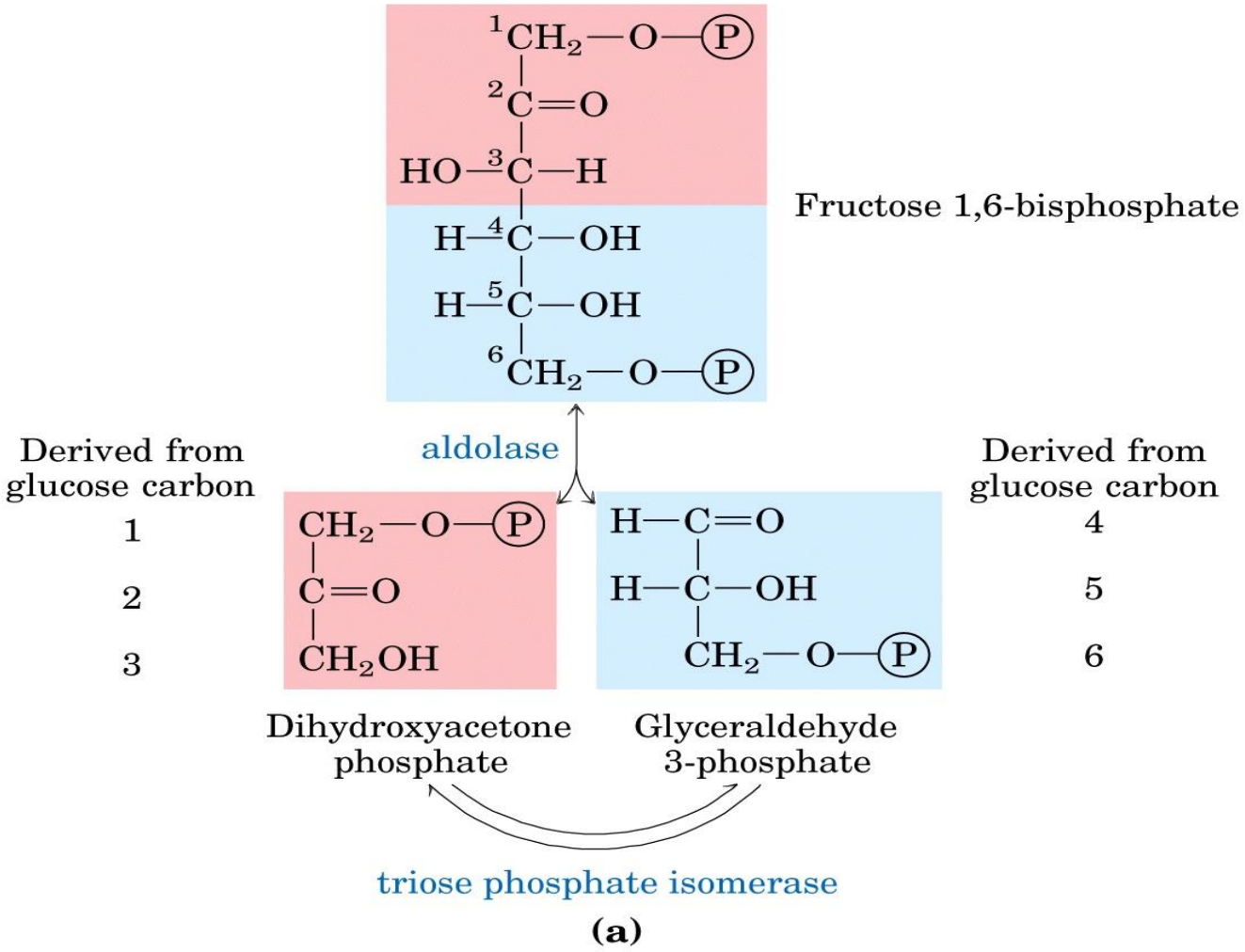
Only glyceraldehyde 3-phosphate is on the direct pathway of glycolysis.

5th Reaction: Isomerization of 3-carbon phosphorylated sugars. catalyzed by *triose phosphate isomerase*.



With this reaction, carbons 1, 2, and 3 of the starting glucose become indistinguishable from carbons 6, 5, and 4, respectively.

Also, the numbering of carbon atoms in glyceraldehyde 3-phosphate is not the same as the numbering of carbons in glucose.

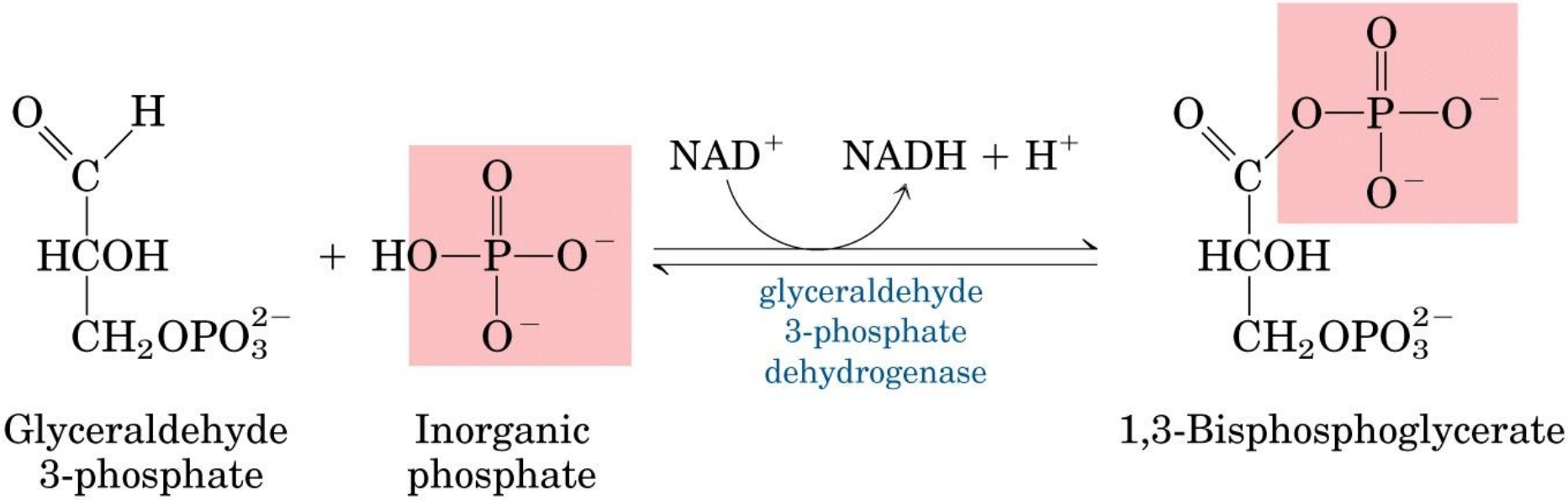


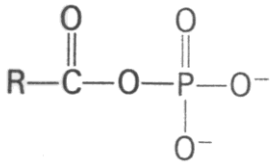
Glycolysis: Stage 2

Stage 1: 1 molecule of glucose → 2 molecules of glyceraldehyde 3-phosphate.

Stage 2: 2 molecules of glyceraldehyde 3-phosphate →
2 molecules of pyruvate

6th Reaction: Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.(dehydrogenase: oxidizes a substrate by transferring one/more protons and a pair of electrons to an acceptor, usually NAD/NADP or flavin coenzyme FAD).



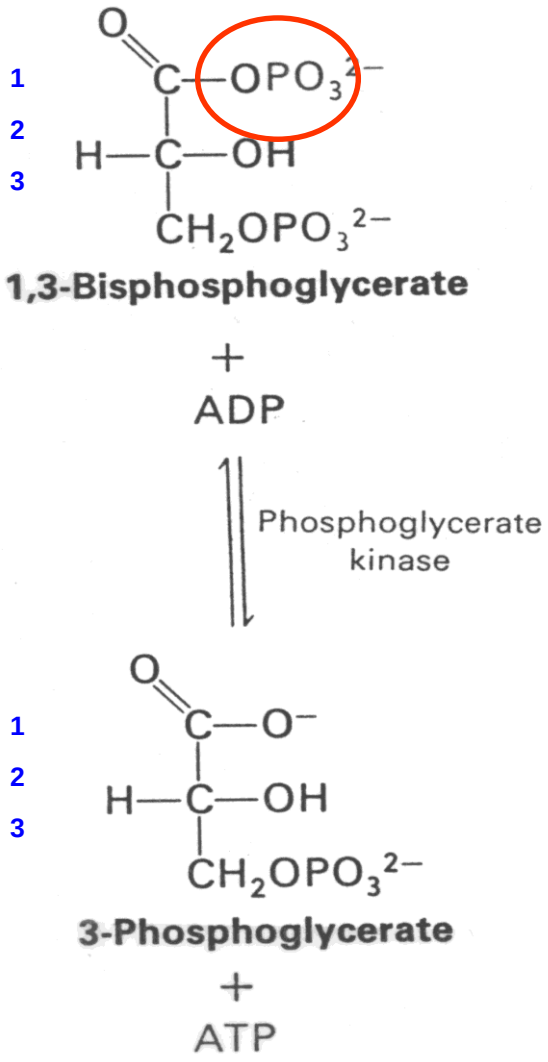


This is a mixed anhydride of phosphoric acid and a carboxylic acid.

Note: The mixed anhydride has a very high free energy of hydrolysis.

The first of the two energy-conserving reactions of glycolysis that will ultimately yield ATP.

7th Reaction: First ATP-generating step. ATP is formed as the phosporyl on the carboxyl group of 1,3-bisphosphoglycerate is transferred to ADP. *"Substrate Level Phosphorylation"*



Note: The consequences of this reaction in combination with the 6th reaction are:

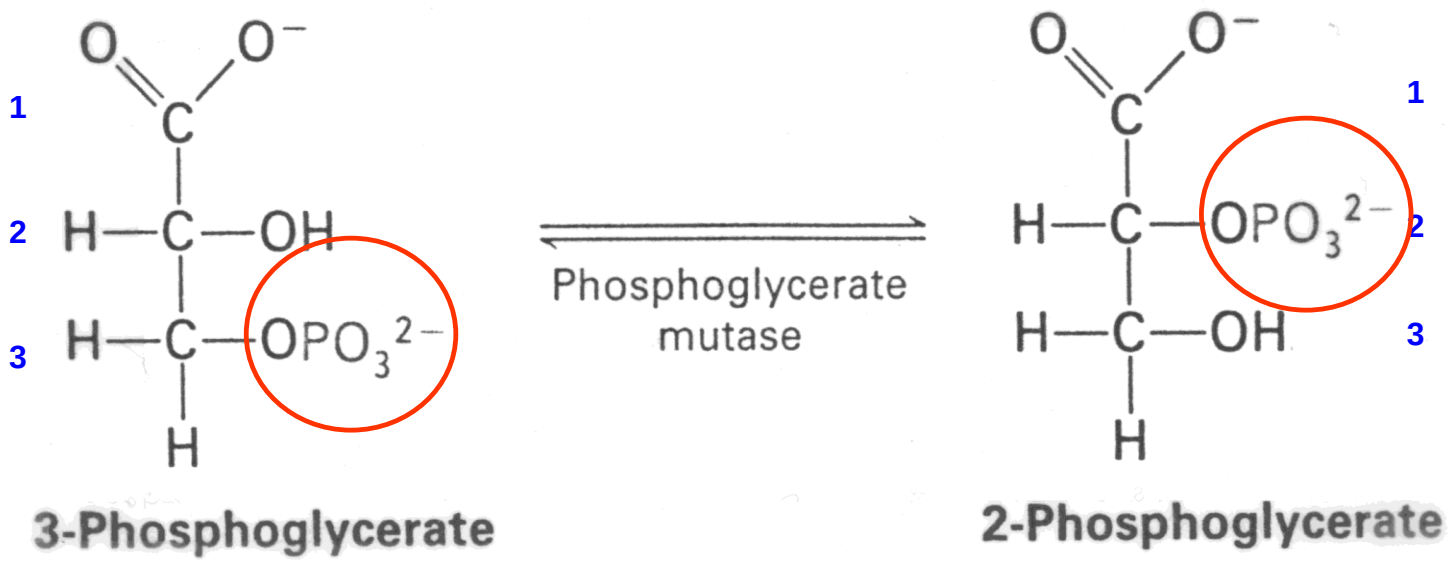
1) An aldehyde is oxidized to a carboxylic acid group.

2) NAD^+ is concomitantly reduced to NADH.

3) ATP is formed from Pi and ADP.

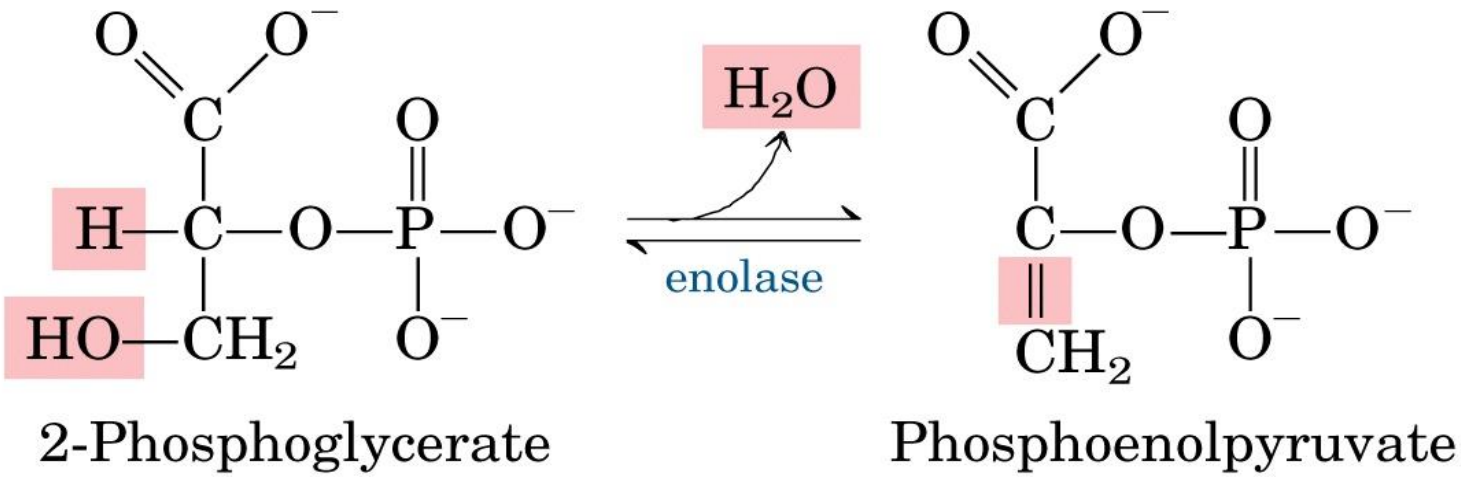
8th Reaction: The phosphoryl group is shifted from the C-3 to the C-2 position of glycerate. Catalyzed by *phosphoglycerate mutase.*
Mg2+ is essential for this reaction.

A mutase transfers a functional group from one position to another on the same molecule.



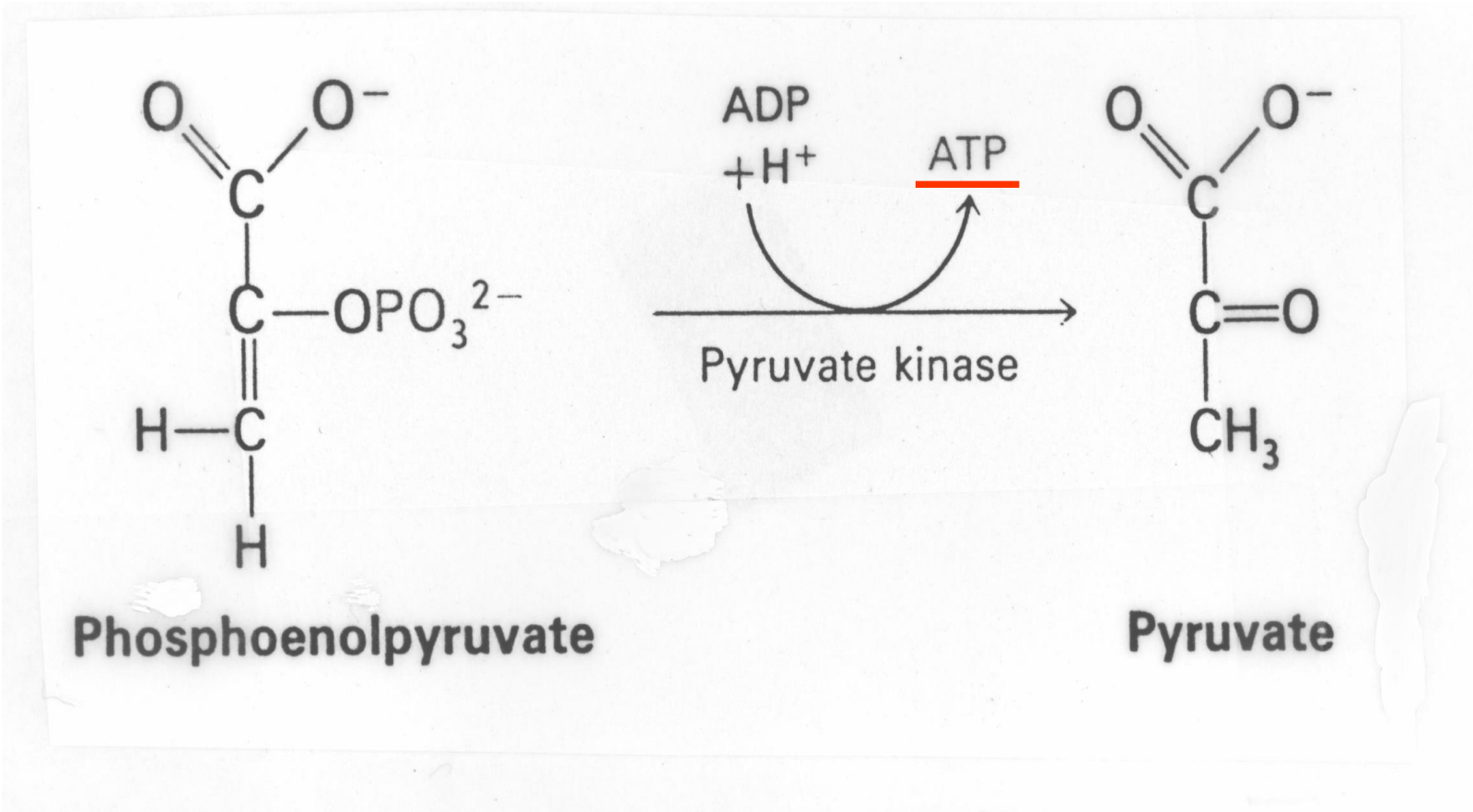
9th Reaction: A dehydration reaction is which water is reversibly removed from 2-phosphoglycerate to from phosphoenolpyruvate.

Catalyzed by *enolase. Mg2+ is required.*



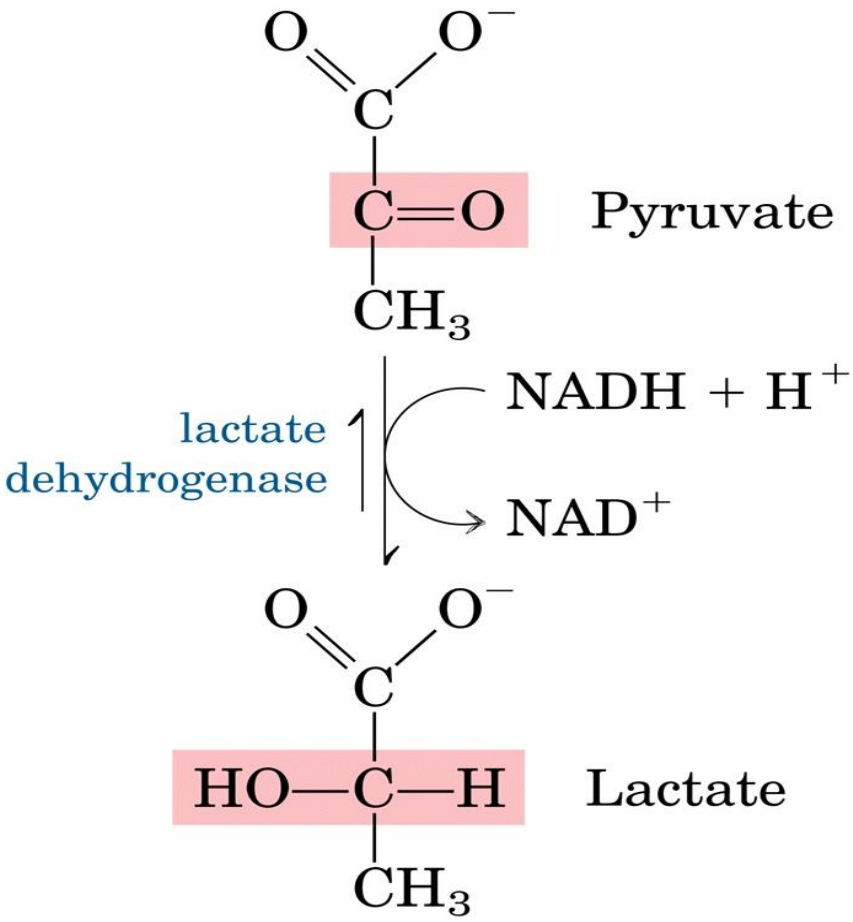
10th Reaction: Transfer of a phosphoryl group from PEP to ADP catalyzed by *pyruvate kinase.*

Irreversible; An important site of regulation in the liver.
K+ and Mg2+ are required.
The second "substrate level phosphorylation".



<u>11th Reaction:</u> Reduction of pyruvate to lactate via the enzyme *lactate dehydrogenase.*

Conversion occurs under partially anerobic conditions, when oxygen is limited (e.g., muscle during intense activity)

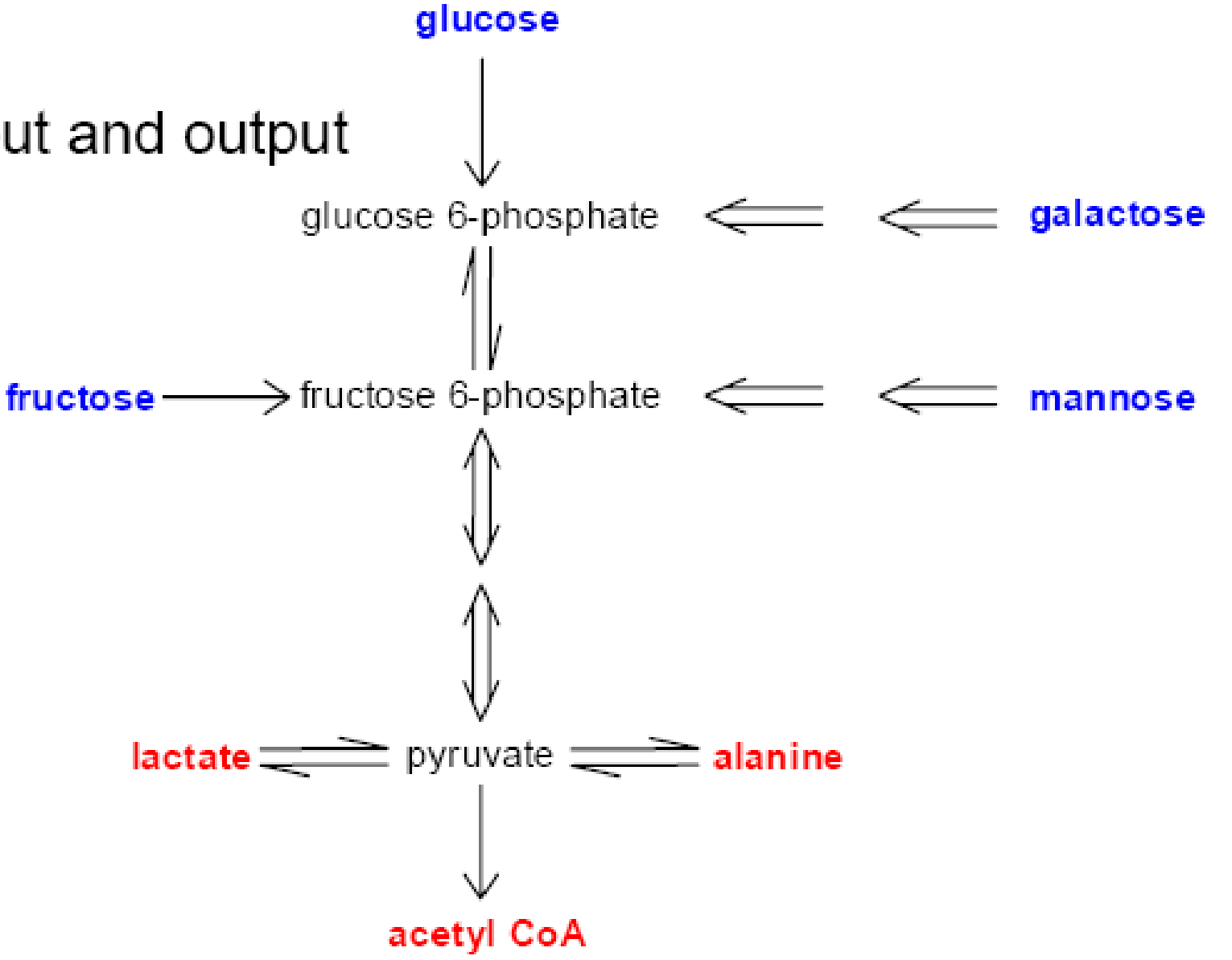


NADH required for this reaction is supplied by the 6th reaction (the dehydrogenation of glyceraldehyde 3-phosphate).

Importantly, under <u>anaerobic</u> conditions, the regeneration of NAD^+ by this step is <u>essential for the continued functioning of glycolysis</u>.

Glycolytic background

- Input and output



Regulation of Glycolysis:

Enzymes catalyzing irreversible reactions are often potential control sites.

In glycolysis regulation occurs at *hexokinase, phosphofructokinase, pyruvate kinase.*

I- Hexokinase

1- glucose (inert)---------glucose (active) for further rxns

2- glucose (non ionic)-------glucose (ionic) trapped in cells no transport system for phosphorylated sugars.

- *Hexokinase* can also phosphorylate hexoses fructose, mannose, and glucosamine, whereas glucokinase cannot.

- First reaction *does not commit glucose to glycolysis*, since glucose-6-phosphate represents a branch point in carbohydrate metabolism. It also enters pentose phosphate pathway and glycogenesis.

-inhibited by its product glucose 6-phosphate. Thus PFK inhibition leads to hexokinase inhibition via the buildup of metabolites.

- ***Glucokinase*** *(type IV hexokinase) :*

Glucokinase is present at high concentration in liver cells (hepatocytes).

[glucose in blood] = 5mM

Km hexokinase = 0.1mM

Km glucokinase = 10mM

(Km = concentration of substrate that gives half-maximal activity)

-is not inhibited by glucose 6-P unlike hexokinase

- At normal blood glucose conc. : hexokinase is fully saturated, glucokinase is not.
- After a meal rich in sugar: in response to high levels of blood glucose glucokinase is induced. To assures that at high conc. , glucose is not wasted glucose→converted to glucose 6-phosphate →synthesis of glycogen.
- At low blood glucose: gives brain and muscle first call on glucose when its supply is limited.

[glucose] high----------glycogen -------storage (glucokinase)

[glucose] low----------glucose to peripheral tissues

LIVER BUFFERS BLOOD GLUCOSE.

II- Phosphofructokinase-1 PFK1 ***:***

The 1st irreversible rxn **unique** to the glycolytic pathway=the committed step. **the most important control point in glycolysis.**

The **rate limiting enzyme** of glycolysis is PFK
The rate limiting enzyme of gluconeogenesis is fructose 1,6 bisphosphatase

PFK regulation :

positive modulators:
ADP/ AMP, substrate fructose 6-phosphate, fructose 2,6-bisphosphate.

negative modulators:
ATP, citrate (an early intermediate in the citric acid cycle), fatty acids, low pH

-High levels of ATP inhibits PFK (Allosteric control)

PFK is a tetrameric enzyme in 2 conformational states R and T that are in equilibrium ATP is both a S. and Inh. Each subunit has two ATP binding sites (S and inh site). The ATP binds the S site in both conformation but it binds the inh. site only in T conformation, F6P binds to the R state of the enzyme. ATP binds to a regulatory site distinct from the catalytic site.

AMP reverse the inhibitory effect of ATP

R (relaxed) **T (tensed)**

F6P, AMP ⟶ **ATP**

-Citrate (glycolysis is a source of c- skeleton for biosynthesis).

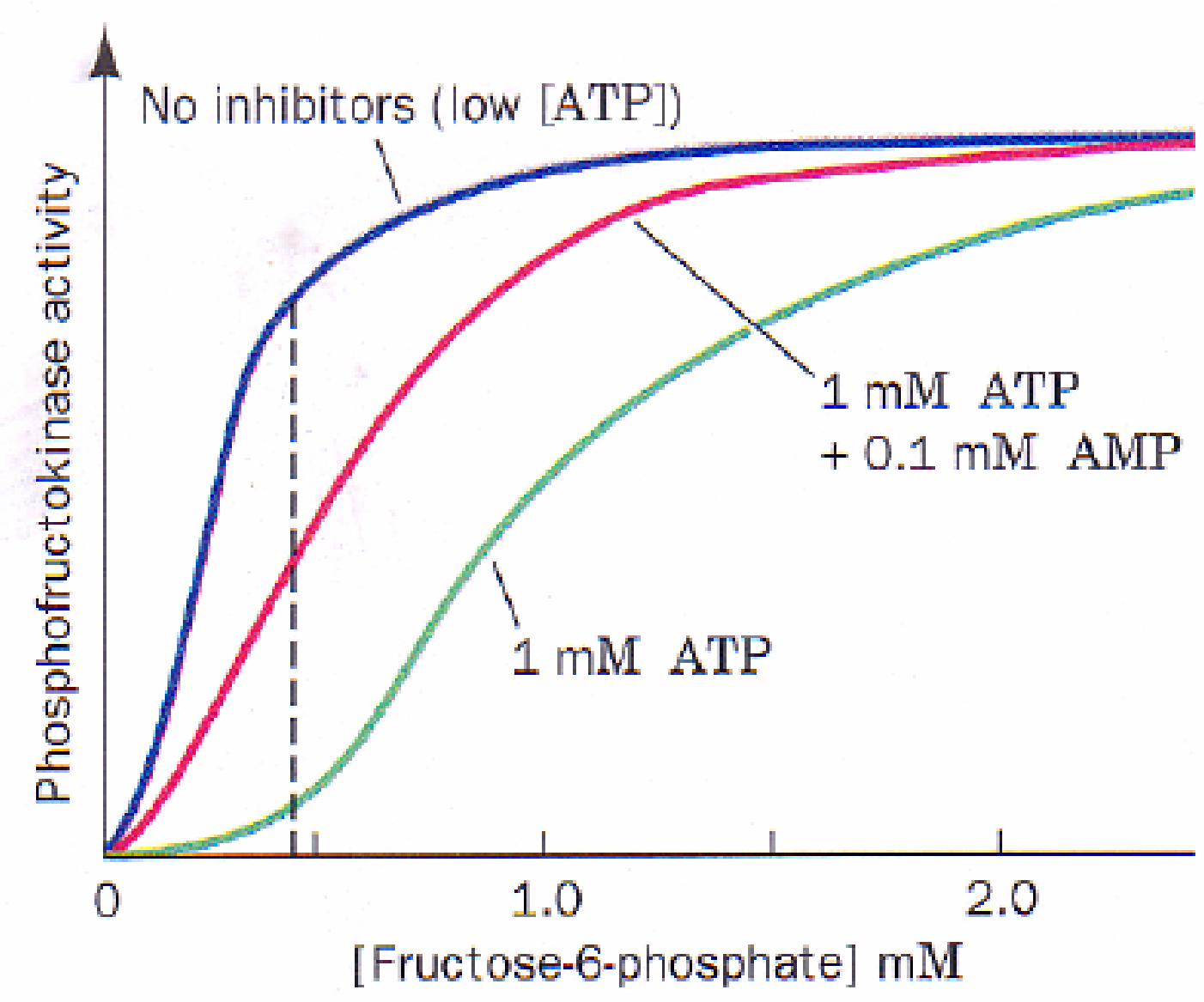
-Low pH inhibits PFK

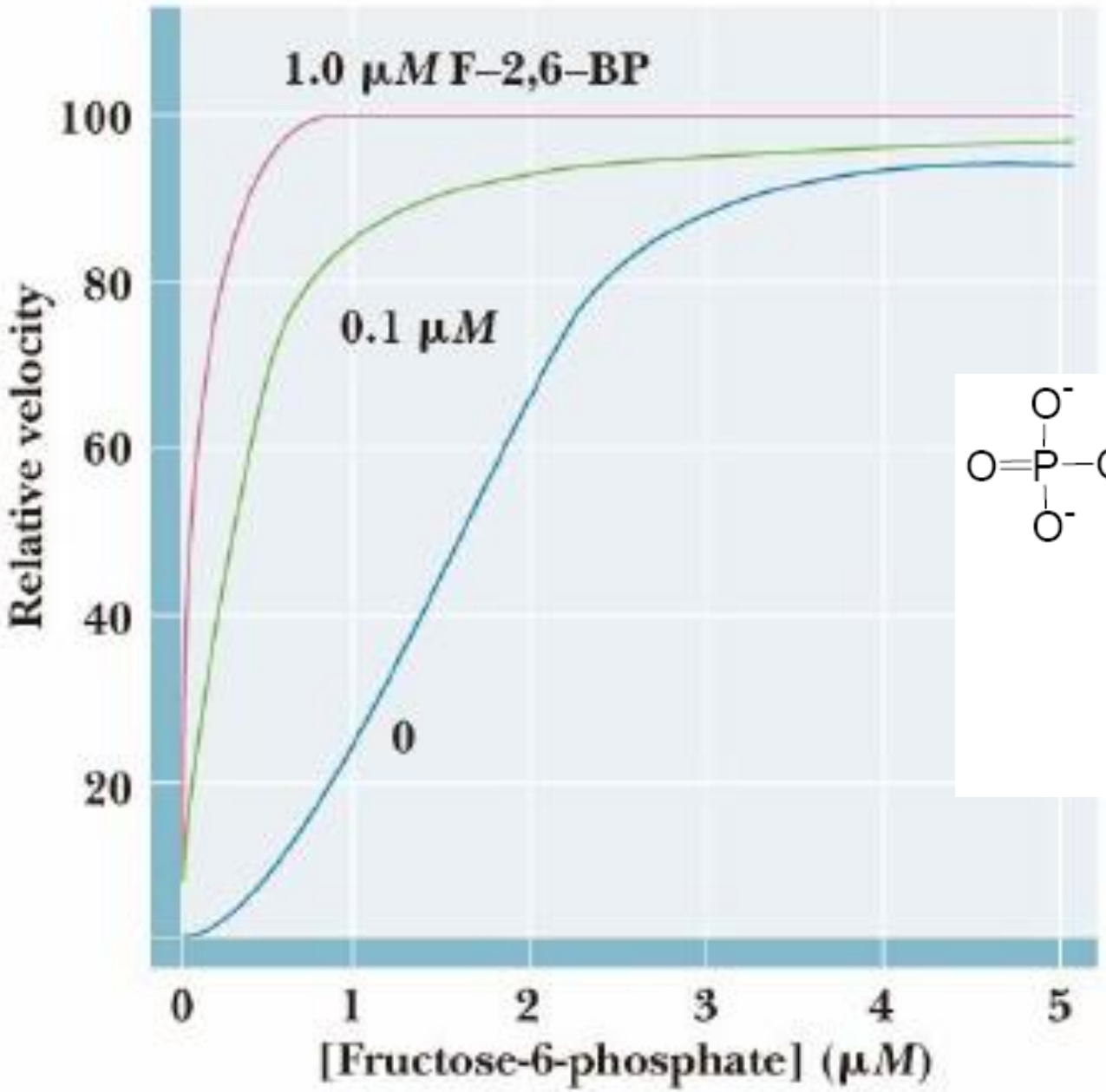
Prevents excessive rise via anaerobic conditions, otherwise -----acidosis (drop in blood pH).

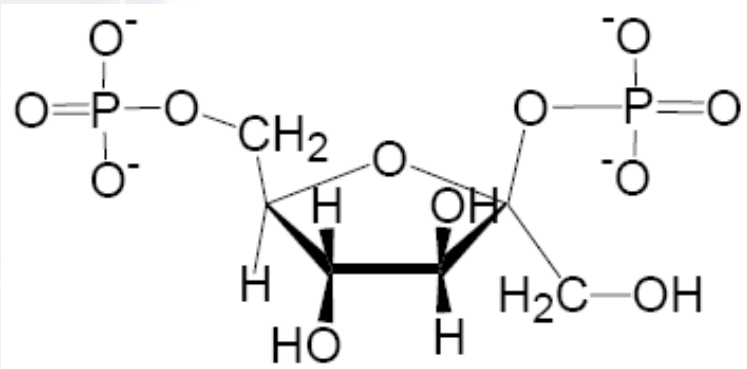
-Fatty acids when catabolized no extensive need for the glycolytic pathway

PFK REGULATION

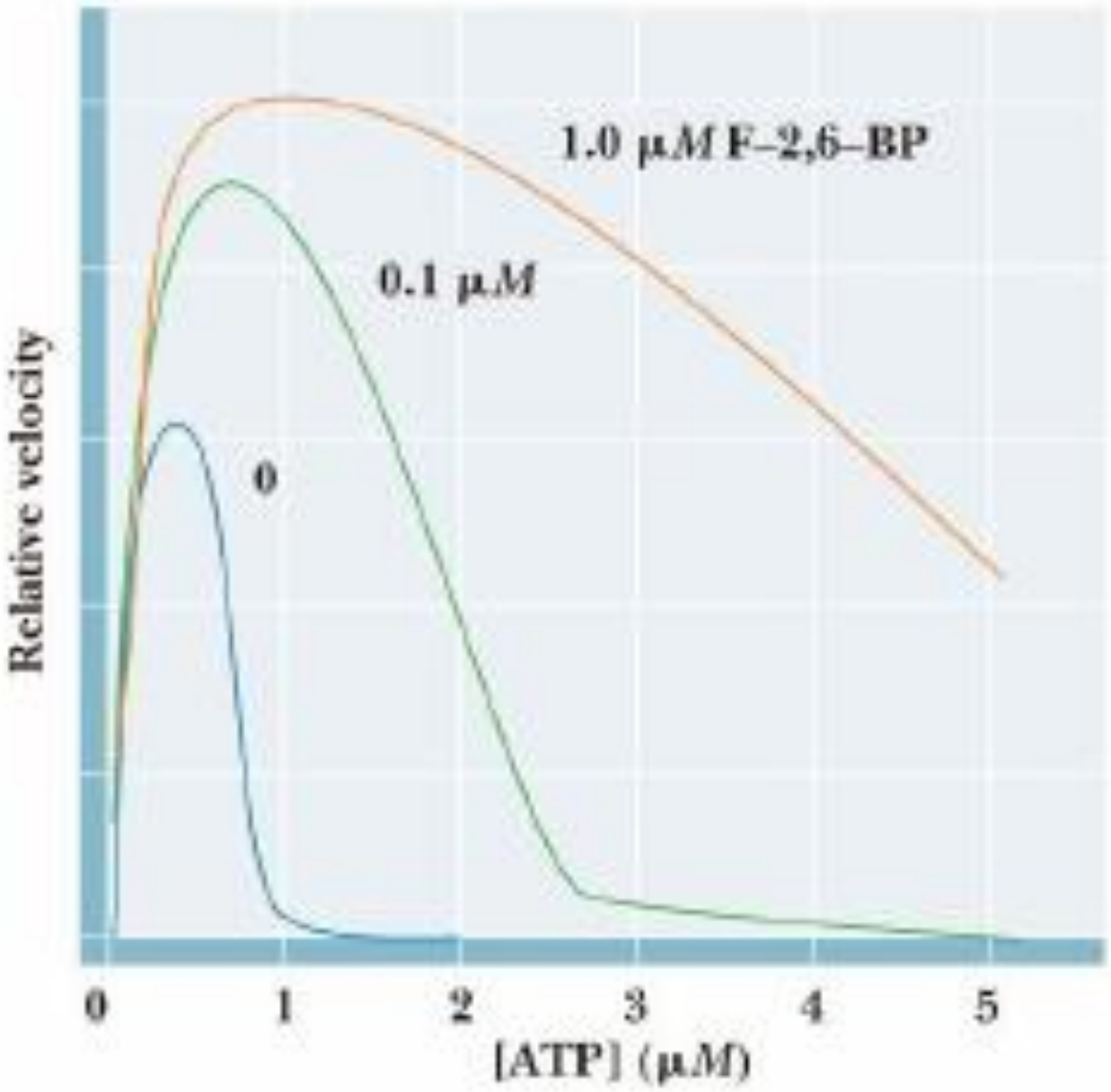
These two conformational states are in equilibrium: T ⟷ R







β-D-Fructose-2,6-bisphosphate


1.0 μM F-2,6-BP
0.1 μM
0
Relative velocity
0
1
2
3
4
5
[ATP] (μM)

Regulation of glycolysis:

Fructose 2,6 diphosphate is not an intermediate (regulation)

Bifunctional enzyme (phosphofructo-kinase2/ fructose 2,6-biphosphatase)
Synthesis and degradation of
fructose-6-phosphate----------fructose 2,6 bisphosphate
fructose 2,6 bisphosphate----------fructose-6-phosphate

cAMP inactivates the kinase
cAMP activates the phosphatase

Pyruvate Kinase a regulatory enzyme :

Activated by Mg2+, Mn2+.
K+ causes a conformational change to give a more active enzyme.

L form in liver , M form in muscle, brain, A type other tissues.

Liver form:
+ve modulators: fructose 1,6-bisphosphate, PEP
-ve modulators: ATP, citrate, alanine, f.a, acetyl CoA
ATP effect similar to that on PFK i.e. ATP binding to the inhibitor site reduces its affinity to PEP.

Control at the synthesis level :

[carbohydrates] increase, PK synthesis increase, cellular levels of PK increase.

Protein kinase: phosphorylates PK-------inactive form

Differential regulation:

Muscle PK (M-type) is not regulated by the same way as the liver type

While **liver PK** is regulated by phosphorylation, allosteric effectors and extracellular conditions to the favor of liver gluconeogenesis.

Muscle form of PK not allosterically regulated
Result that muscle glycolysis proceed in accord with intracellular needs.

There are 5 isozymic forms of ***lactate dehydrogenase.(LDH)***

Isozymes: multiple forms of a given enzyme that catalyze the same reaction but differ in kinetic or regulatory properties.

LDH-1 (4H) in the heart

LDH-2 (3HM) endothelial system, spleen, lymph nodes, RBC.

LDH-3 (2H2M) lungs

LDH-4 (1H3M) kidneys

LDH-5 (4M) liver, muscle

H form (heart) and M form (muscle). Designated H_4, H_3M_1, H_2M_2, etc.

Each LDH isozyme contains **4 copies of two different polypeptides**.

All have the same M.wt =33,500 each isozyme 134,000

Differ in their affinity for substrate and sensitivity to allosteric inhibition.

Test for tissue damage , diseases (heart attack, cancer ,HIV)

H_4: higher affinity for substrate; allosterically regulated.

Designed to oxidize lactate to pyruvate which can be used by the heart as an aerobic fuel source.

M_4: optimized to convert pyruvate to lactate in muscle; allows glycolysis to continue under anaerobic conditions.

The liver is a vital part of homeostasis:

When blood glucose is high (hyperglycemia):
Extra glucose-6-phosphate ------glycogen
And hexokinase is inhibited.

During hypoglycemia:
Glycogen ------glucose-6-phosphate--------glucose
Converted by glucose-6-phosphatase to glucose
Importance to maintain blood sugars levels during fasting
And critical for neurons (use only glucose as an energy source)

- **In cancer**
- Malignant rapidly-growing tumor cells typically have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin.
- There are two common explanations.

  1- The classical explanation is that there is poor blood supply to tumors causing local depletion of oxygen.

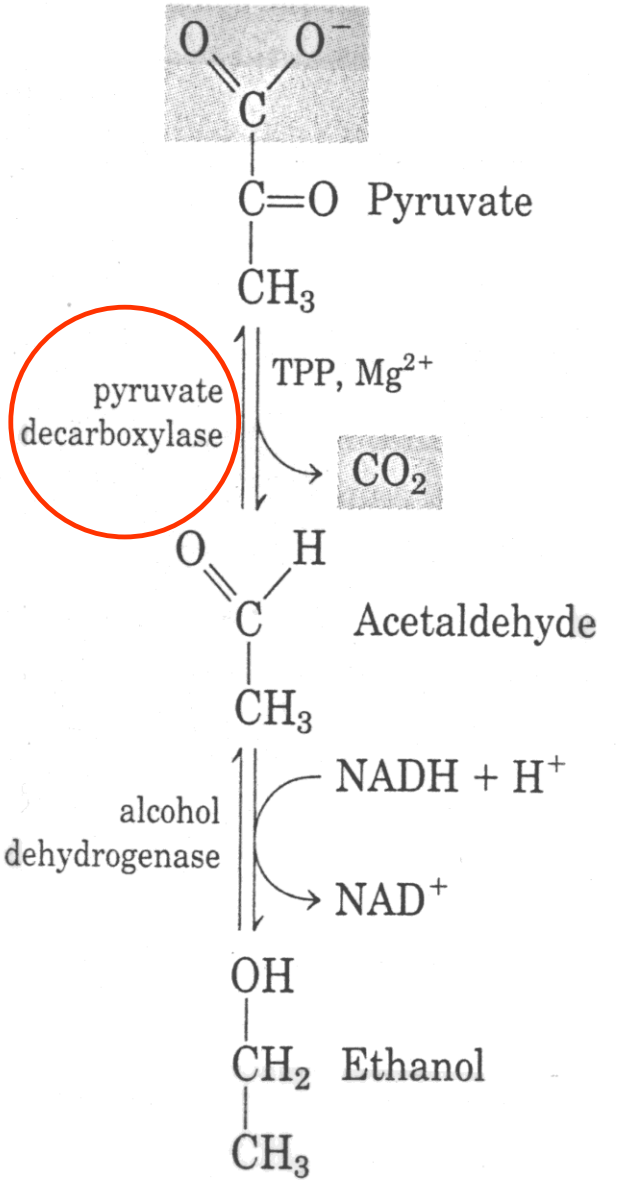
  2- High glycolytic rates due to an overexpressed hexokinase responsible for driving the high glycolytic activity.

This phenomenon was first described in 1930 by Otto Warburg, and hence referred to as the Warburg effect.

Alcoholic Fermentation

The sequence of reactions from glucose to pyruvate is similar in all organisms.

However, in yeast and several other microorganisms ethanol is formed from pyruvate via the following 2 reactions:



Note:
the CO_2 produced via pyruvate decarboxylation in yeast is responsible for the carbonation of champagne.

In baking the CO_2 released when yeast is mixed with a fermentable sugar causes the dough to rise.