# Carbohydrate metabolism
# Glycolysis

By

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# Objectives

- Definition of metabolism.
- Describe anabolic and catabolic pathways.
- Define glycolysis
- Phases of glycolysis
- Aerobic & anaerobic glycolysis
- Difference between hexokinase & glucokinase
- Energy production during glycolysis
- Regulation of glycolysis.
- Fate of pyruvate

# *METABOLISIM*

- **Metabolism** is defined as:
  - Interconversion of chemical compounds in the body
  - The pathway taken by individual molecules
  - Their inter-relationships
  - Mechanism that regulate the flow of metabolites through the pathways.
  - **Metabolite**: The substances obtained on metabolic activity like substrate , intermediate or a product in the metabolic reaction.

# Introduction to Metabolism

- Complex substances are broken down for energy, metabolites, structural components, etc.

- Cells must synthesize new complex substances.

- Thousands of such reactions are occurring simultaneously in a single cell.

- The rate of metabolic pathway can respond to regulatory signals arising within the cells.

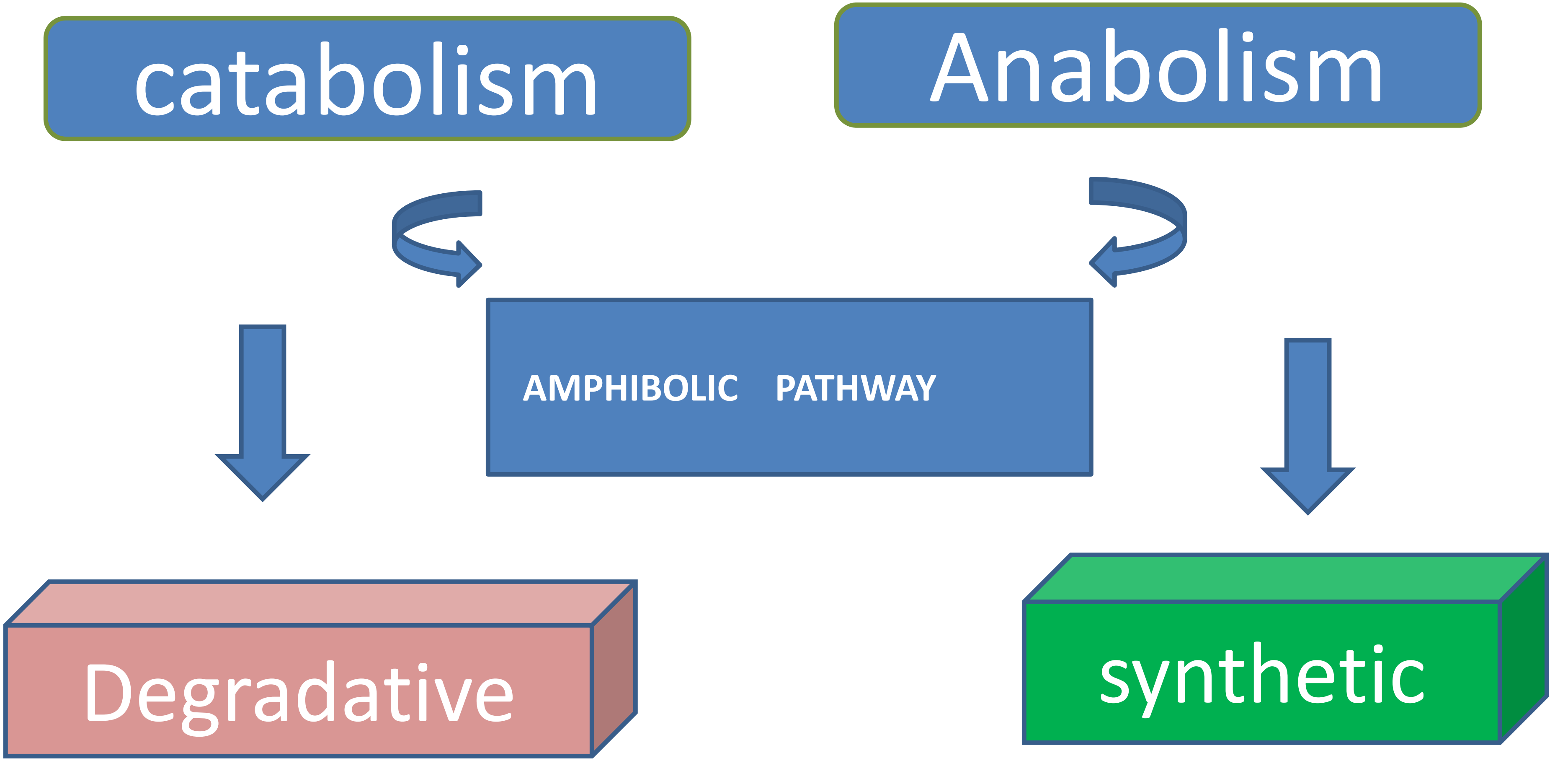
- Regulatory signals include hormones, neurotransmitters and the availability of nutrients.

Three major pathways:

**ANABOLIC PATHWAY**

**CATABOLIC PATHWAY**

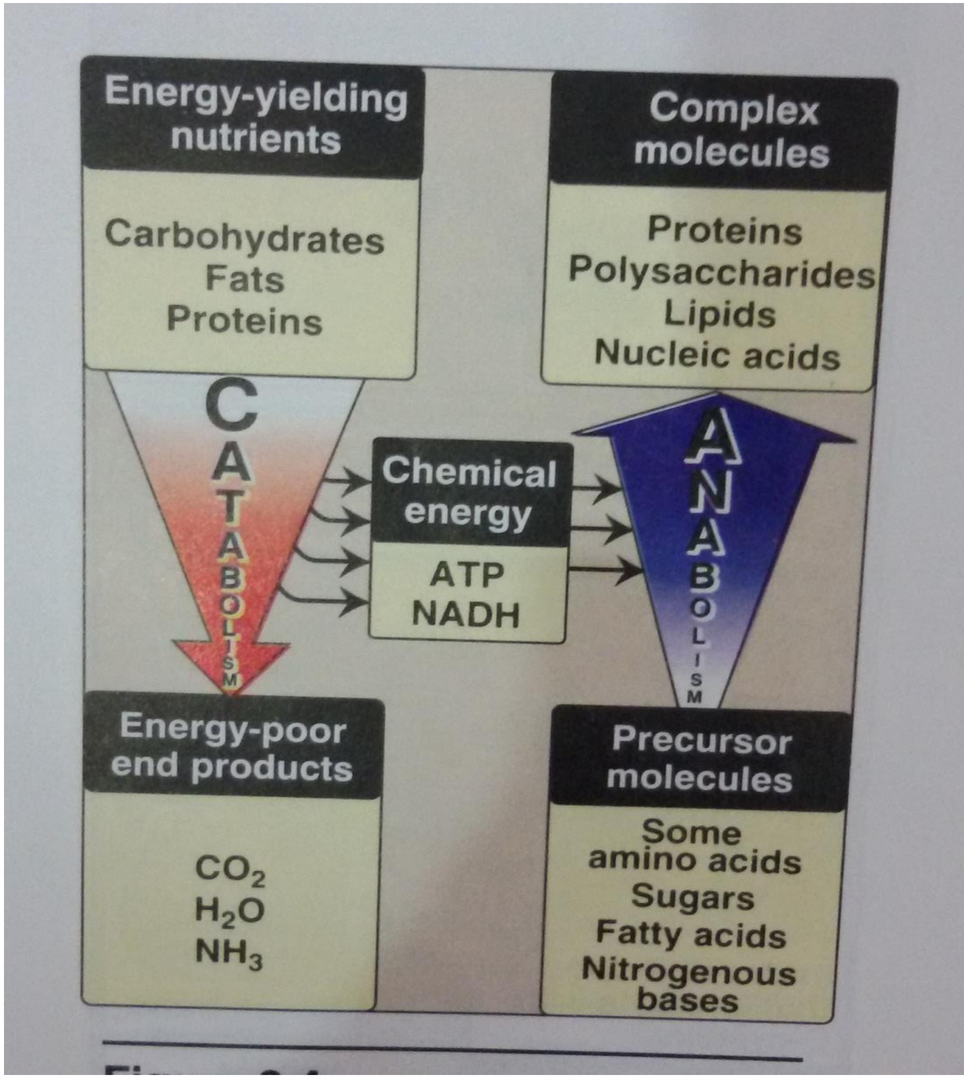
**AMPHIBOLIC PATHWAY**



# ANABOLIC PATHWAY

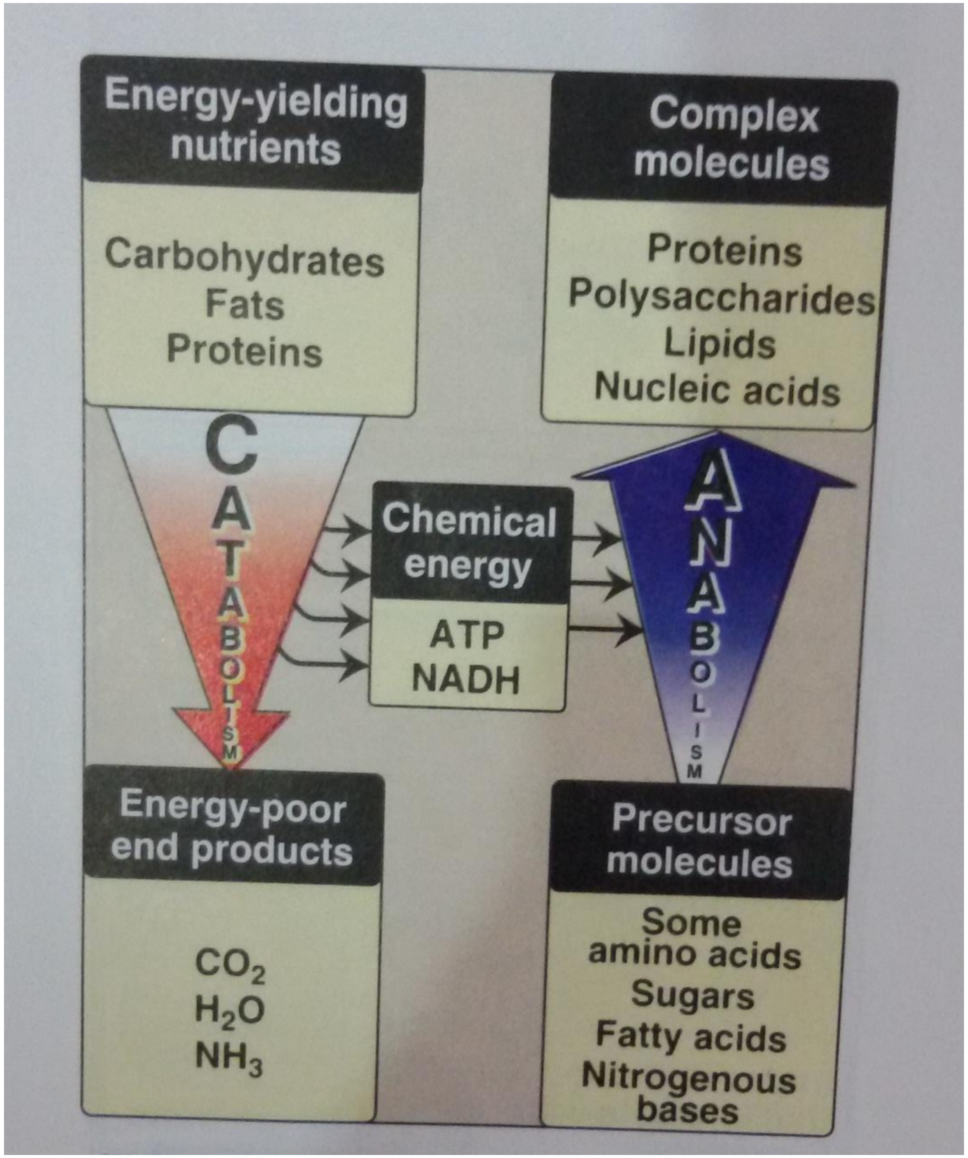
- All the reactions concerned with the synthesis of compounds (metabolites) used by the cell or organism.

- Small molecules combine to form complex molecules.
- Example: synthesis of polysaccharide from glucose and glycogen.
- Anabolic reactions are endergonic require energy provided by breakdown of ATP to ADP + Pi



# *CATABOLIC PATHWAY*

- All the reactions concerned with generating and storing energy for the needs of the cell and organism.
- Involved in the breakdown of larger molecules
- Commonly involving oxidative reactions
- They are exothermic producing reducing equivalents ,ATP



Stage-1

Hydrolysis of complex molecules to their component building blocks

Stage -2

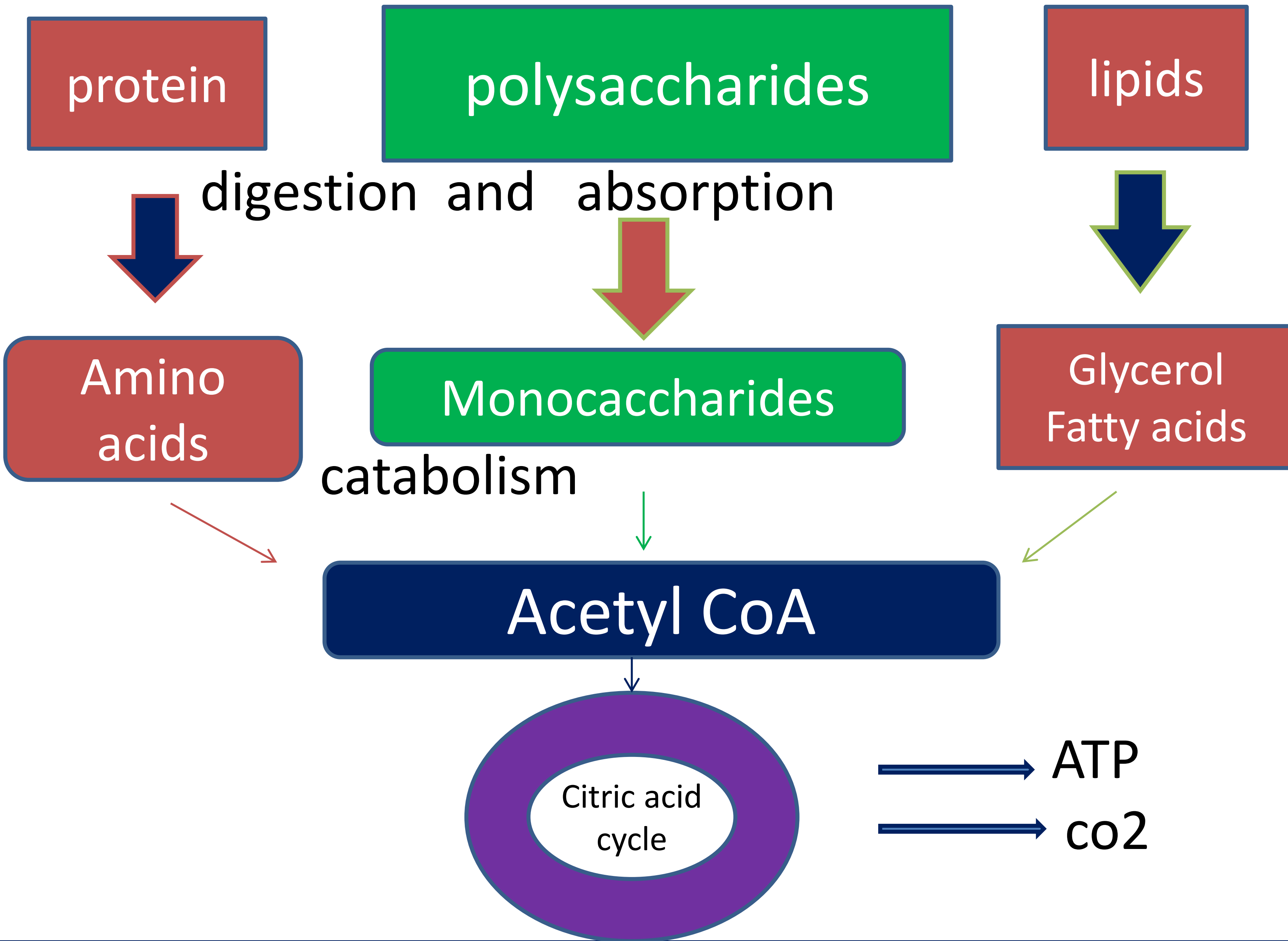
conversion of building blocks to acetyl CoA
(Or other simple intermediate)

Stage-3

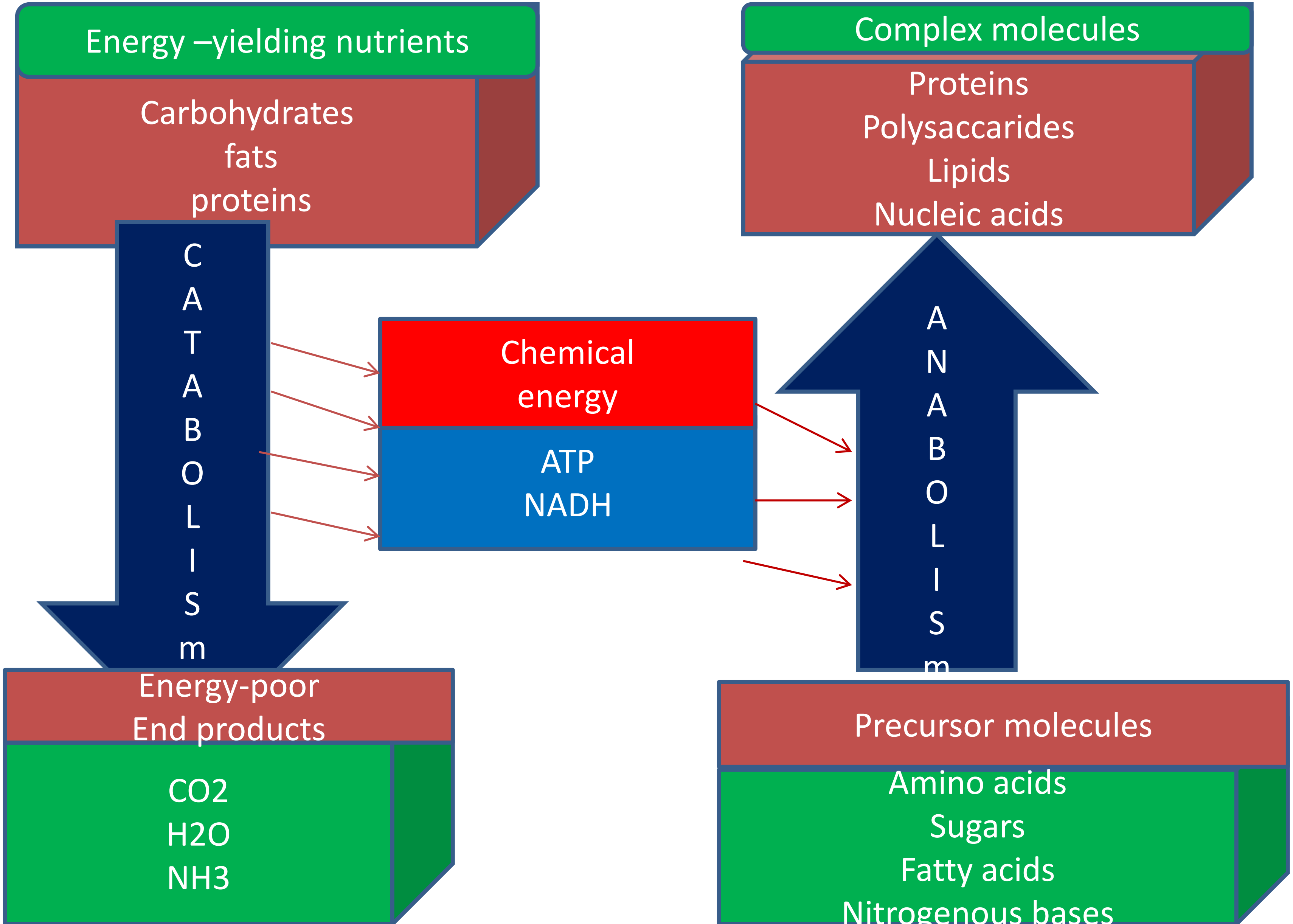
Oxidation of acetyl CoA; oxidative phosphorylation

# Stages of catabolism

# Stages of catabolism

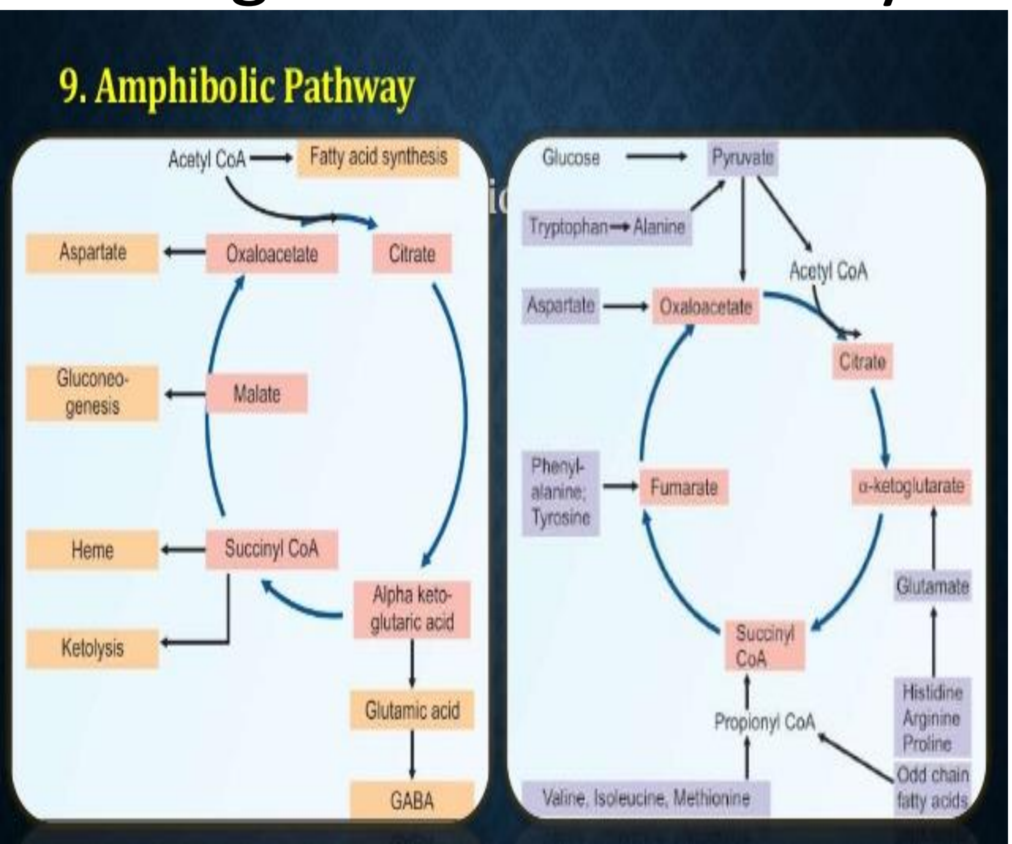


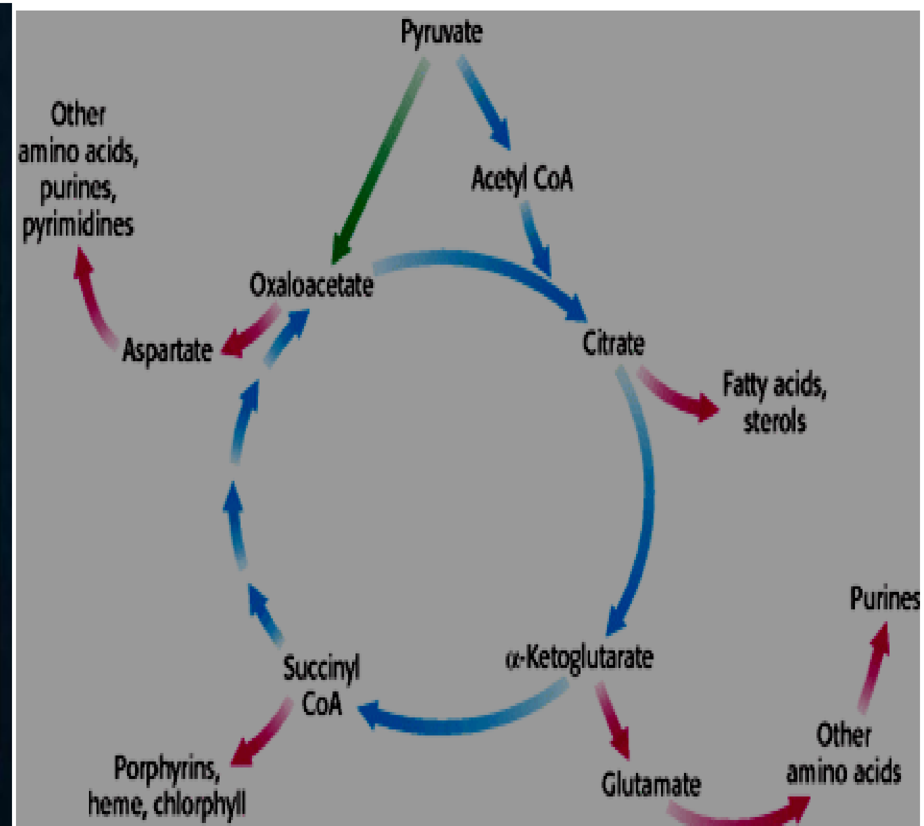
# Comparison of catabolic and anabolic pathway



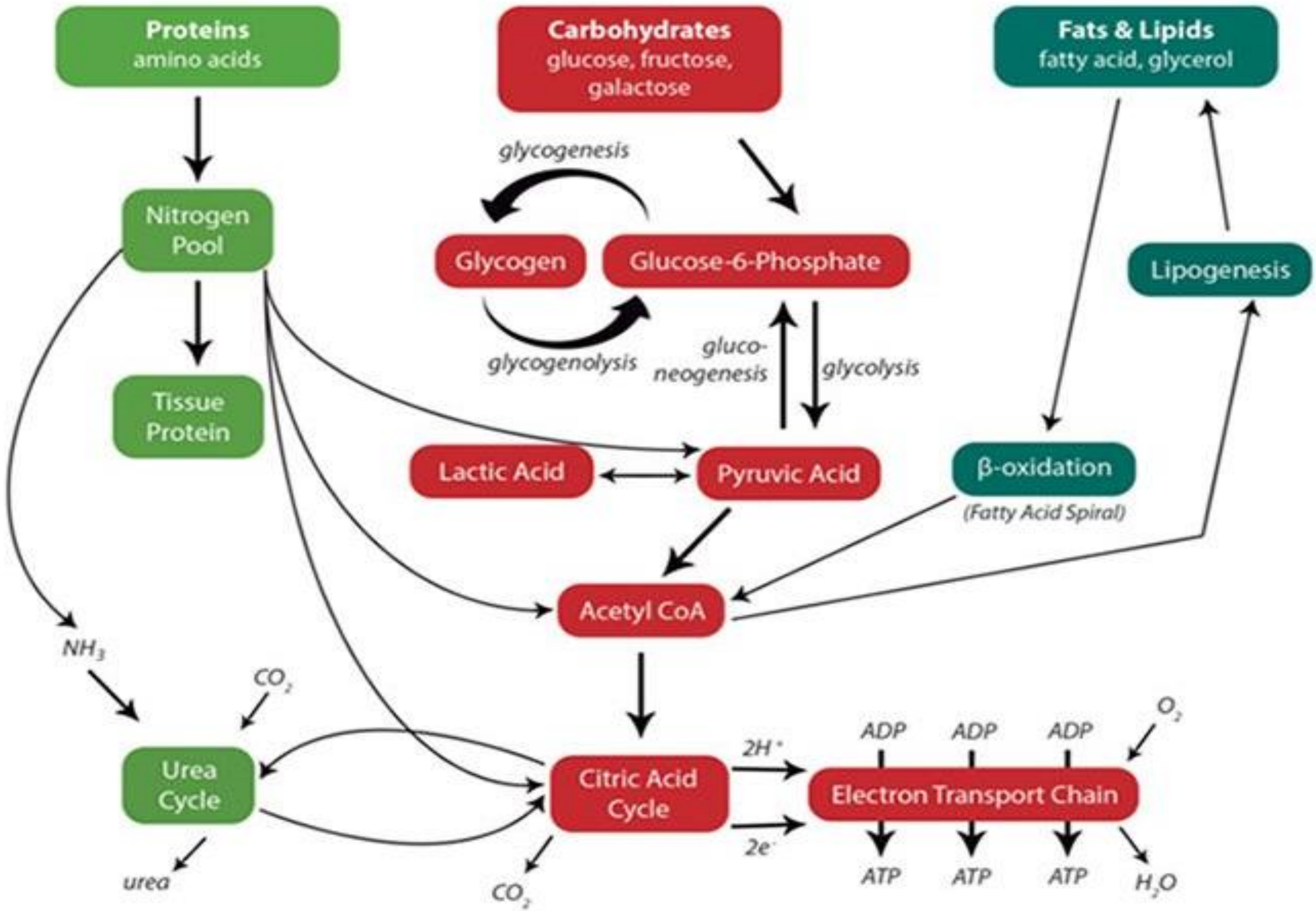
# *AMPHIBOLIC PATHWAY*

- Describes a biochemical pathway that involves both catabolism and anabolism.
- eg. The Citric Acid Cycle.



# Metabolism Summary



# Types of metabolic reaction

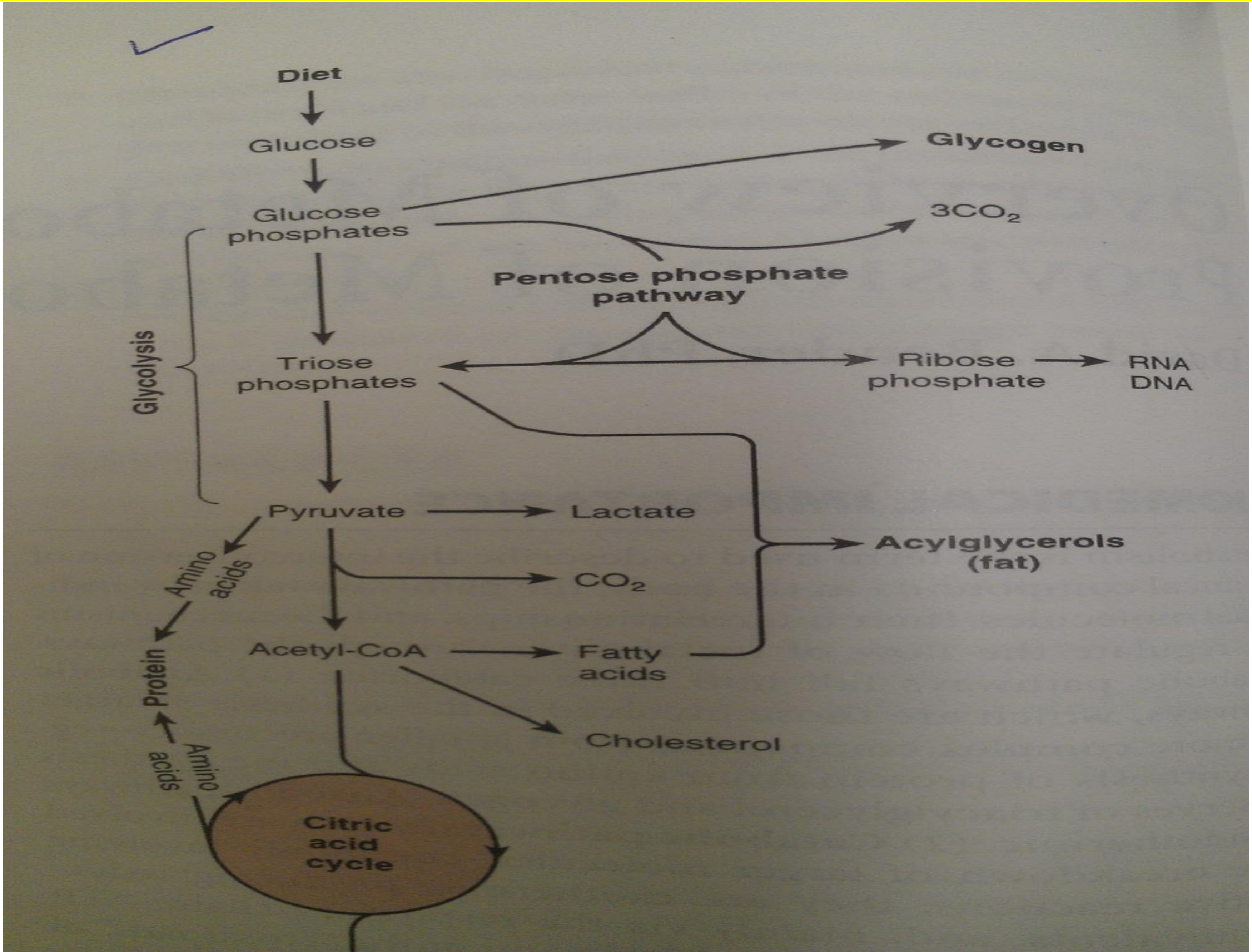
- 1: Oxidation-reduction.
- 2: Group transfer.
- 3: Rearrangement and isomerisation.
- 4: Make and break of carbon-carbon bonds.

These reaction are catalysed by specific enzymes-more than 2000 known so for.

# *GLYCOLYSIS*

- Oxidation of glucose or glycogen to pyruvate & lactate.
- Major pathway for glucose metabolism
- Pathway is for the breakdown of glucose to provide energy ( ATP )
- Glycolysis is at the hub of CHO metabolism as all sugars can ultimately be converted to glucose.
- It occurs in the cytosol of all tissues.
- Functions either aerobically or anearobically _________ depending on presence of O2.

# Carbohydrate metabolism overview

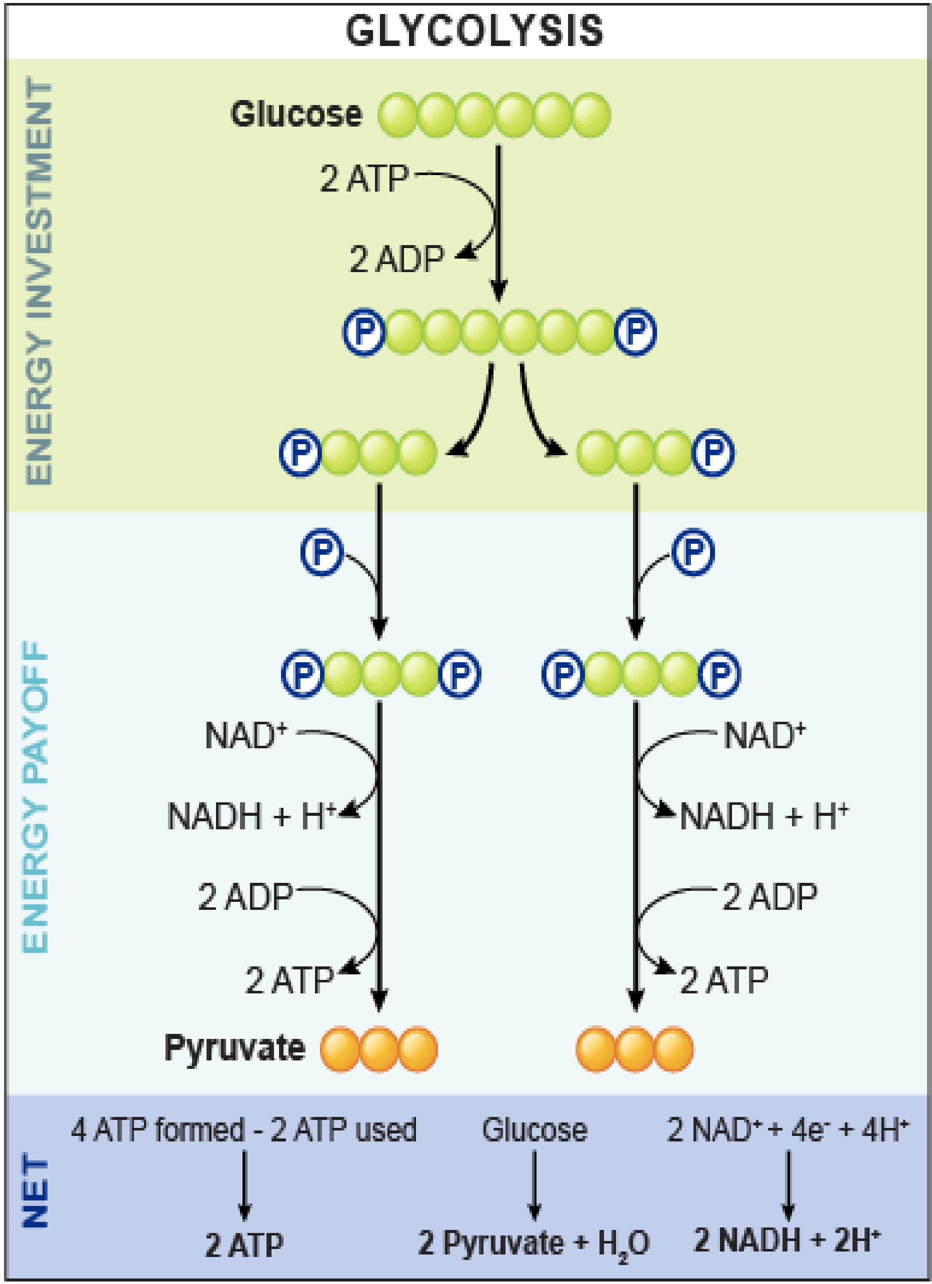


# Glycolysis

- Glycolysis means splitting sugars :
- In glycolysis, glucose(six carbon sugar) split into molecule of 3-carbon sugar.

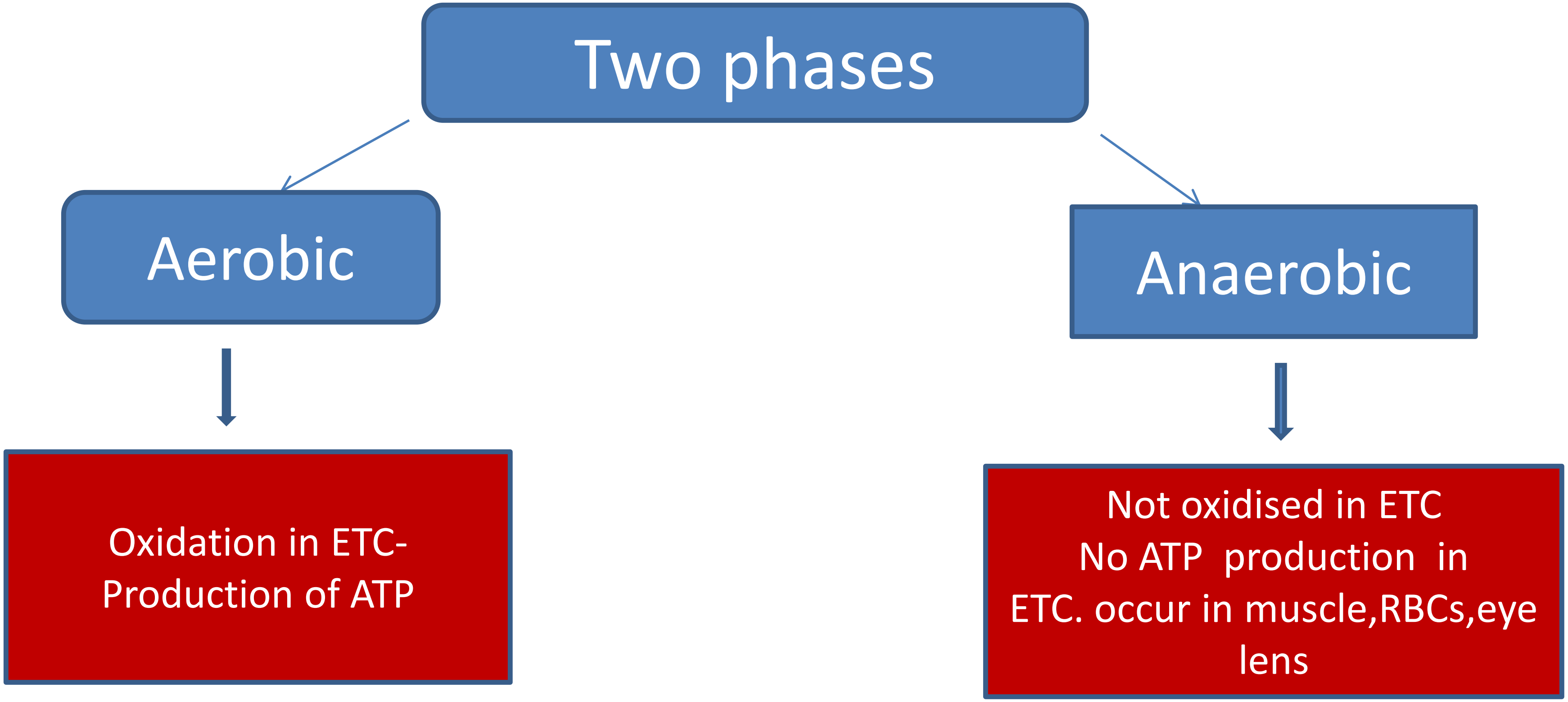
Glycolysis yields

- Two molecules of ATP(free energy containing molecule).
- Two molecules of pyruvic acid,
- Two :high energy: electron carrying molecules of NADH.
- All carbohydrates to be catabolised must enter glycolytic pathway.



# Phases of GLYCOLYSIS

- ***Aerobic glycolysis***
- ***Pyruvate*** is the end product of aerobic glycolysis.
- Oxidation is carried out by dehydrogenases & reducing equivalent is transferred to NAD. Reduced NAD is oxidized in ETC producing ATP.

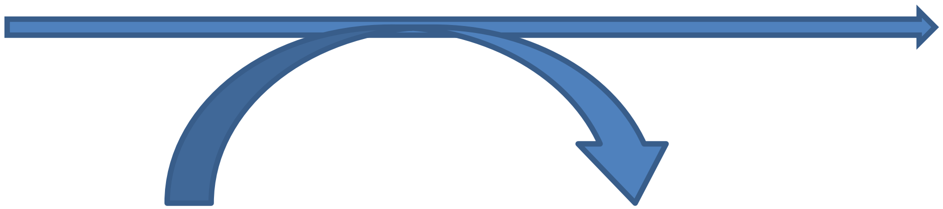


# Anaerobic Glycolysis

- Occur in
- Sk, muscles during strenuous exercise(anoxic condition).
- In erythrocytes, cornea &lens (absent mitochondria).

lactate dehydrogenase

- Pyruvate → lactate

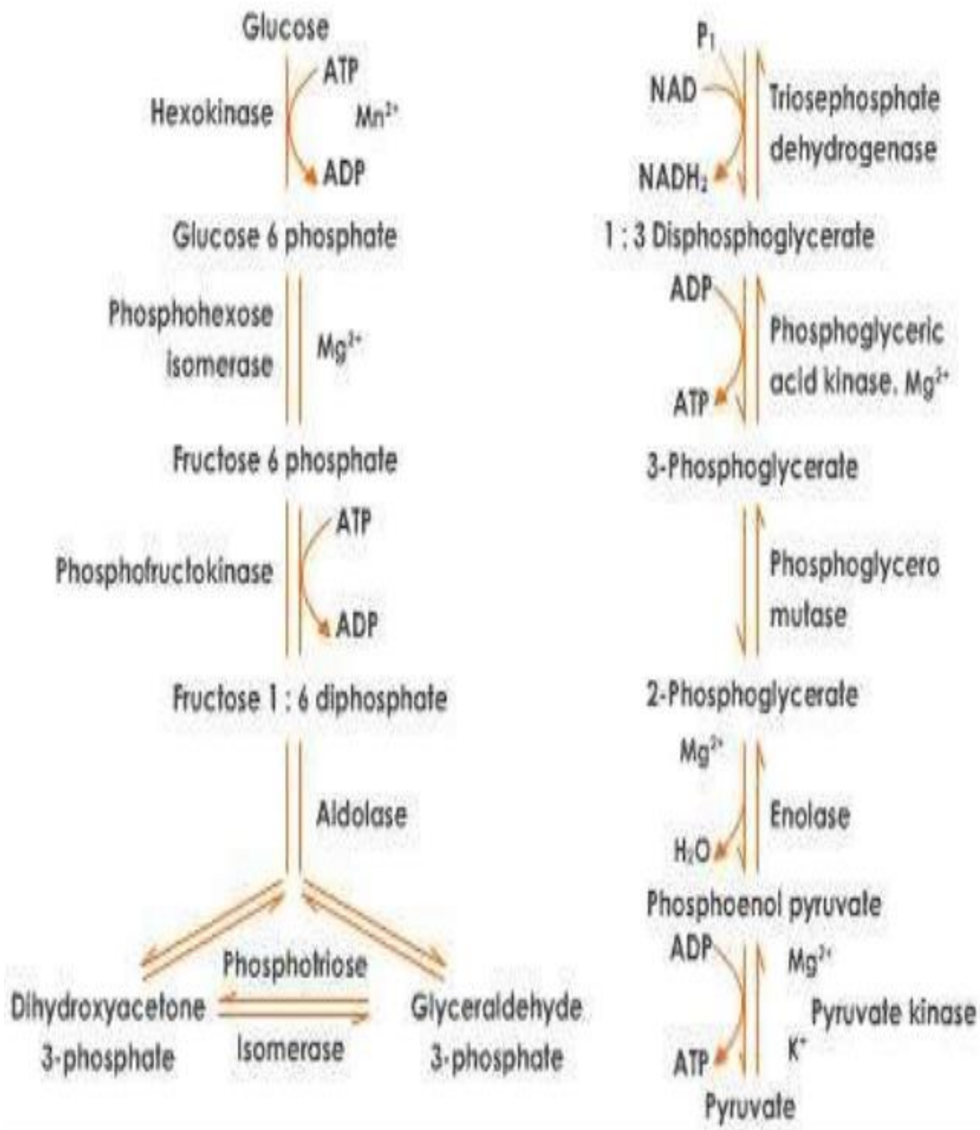


NADH+$H^+$ NAD$^+$

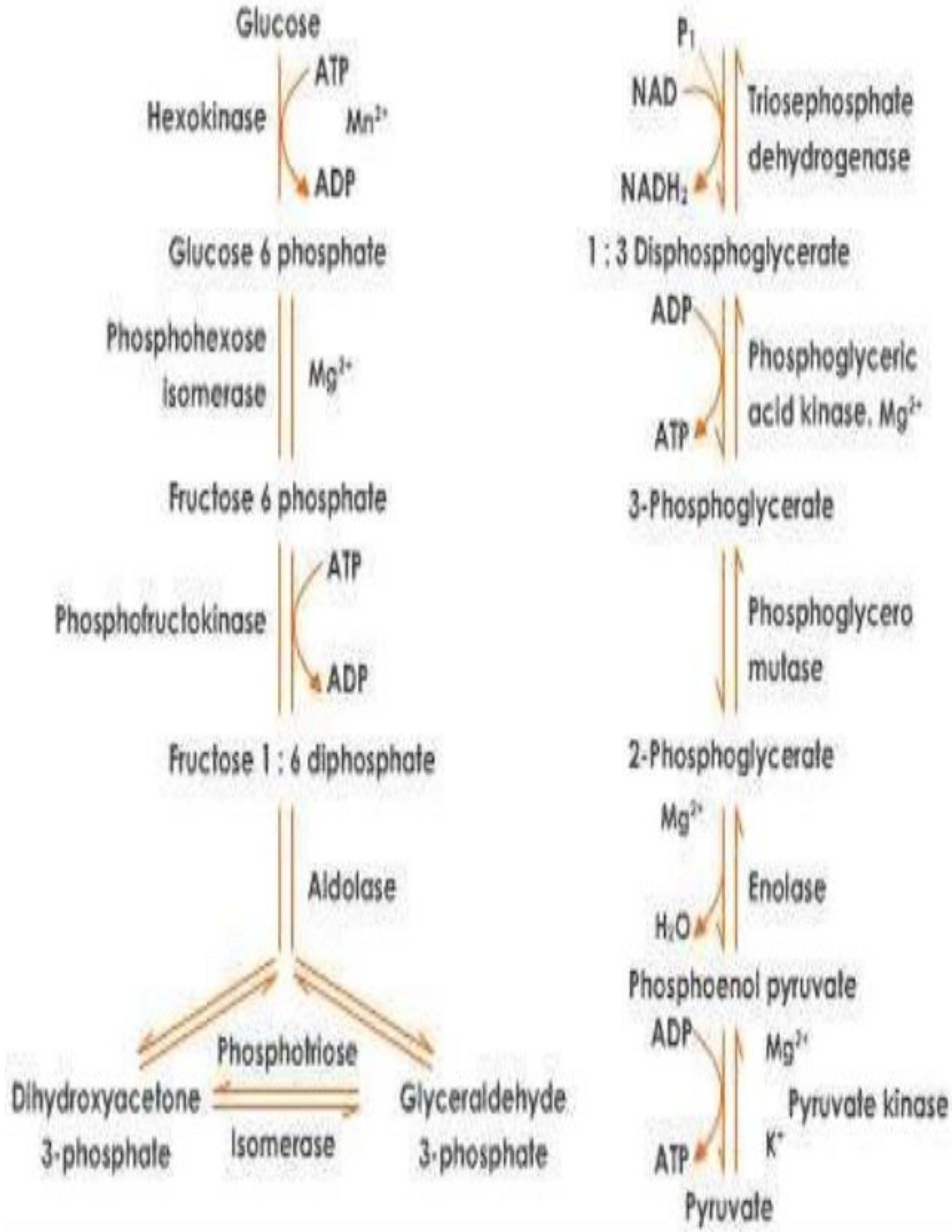
- NADH utilized in this step is obtained from reaction catalyzed by glycraldehyde-3-phosphate dehydrogenase.

# ENZYMES OF GLYCOLYSIS

- ***KINASES :*** transfers phosphate group from the ATP
- ***ISOMERASES :*** converts one isomer to anther
- ***Dehydrogenase :*** removes hydrogen by oxidation. Usually requires NAD or FAD as cofactors/co-substrates

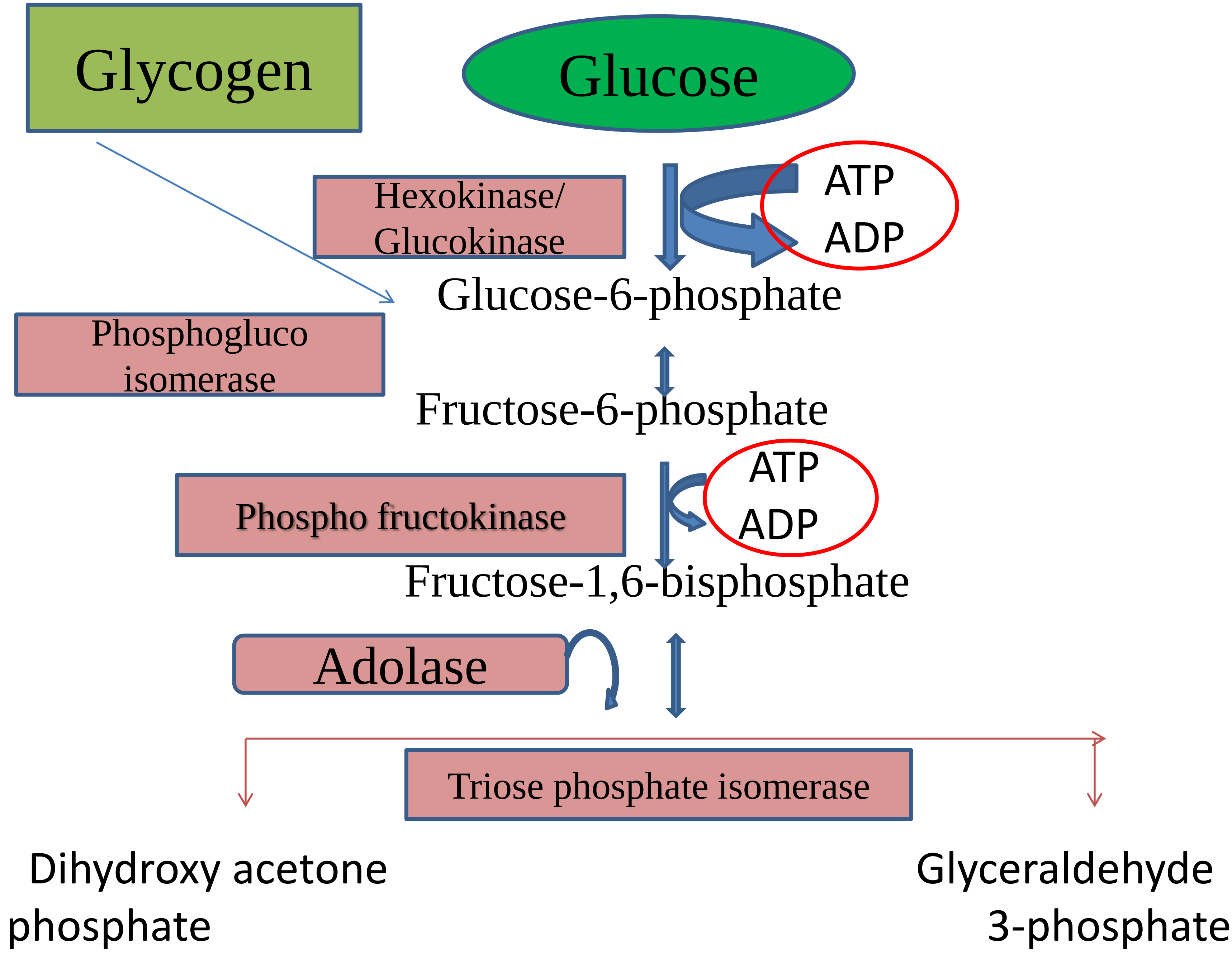


- ***Mutase :*** these are transferring enzymes. Transfers phosphate groups to another position on sugars.
- ***Enolase :*** converts C=C group to alcohol.no change in oxidation state



# Glycolysis has two stages

- Ist preparatory phase is energy requiring phase
- First five reactions corresponds to this phase where phosphorylated form of glucose & fructose are synthesized at the expense of two ATP per glucose molecule.


Glycogen
Glucose
Hexokinase/
Glucokinase
ATP
ADP
Glucose-6-phosphate
Phosphogluco
isomerase
Fructose-6-phosphate
Phospho fructokinase
ATP
ADP
Fructose-1,6-bisphosphate
Adolase
Triose phosphate isomerase
Dihydroxy acetone
phosphate
Glyceraldehyde
3-phosphate

# Phase I. Energy Investment.

1- Glucose is phosphorylated. This phosphorylation at the expense of an ATP *commits* the glucose to this pathway.

Enzymes = ***Hexokinase or Glucokinase***

# Hexokinase vs Glucokinase

| Characteristic | Hexokinase | Glucokinase |
|---|---|---|
| Tissue distribution | All tissues | Liver & pancreatic β cells |
| Km | Low (high affinity) | High (low affinity) |
| Vmax | Low | High |
| Effect of insulin | No effect | Inducible by insulin |
| Substrate specificity | Glucose, fructose & galactose | Glucose*** |
| Allosterically inhibited by glucose-6P | Yes | No |
| Physiological role | Basal levels of glucose-6P for glycolysis & ATP production | Accumulation of high intracellular levels of glucose-6P for conversion to glycogen & TAGs |

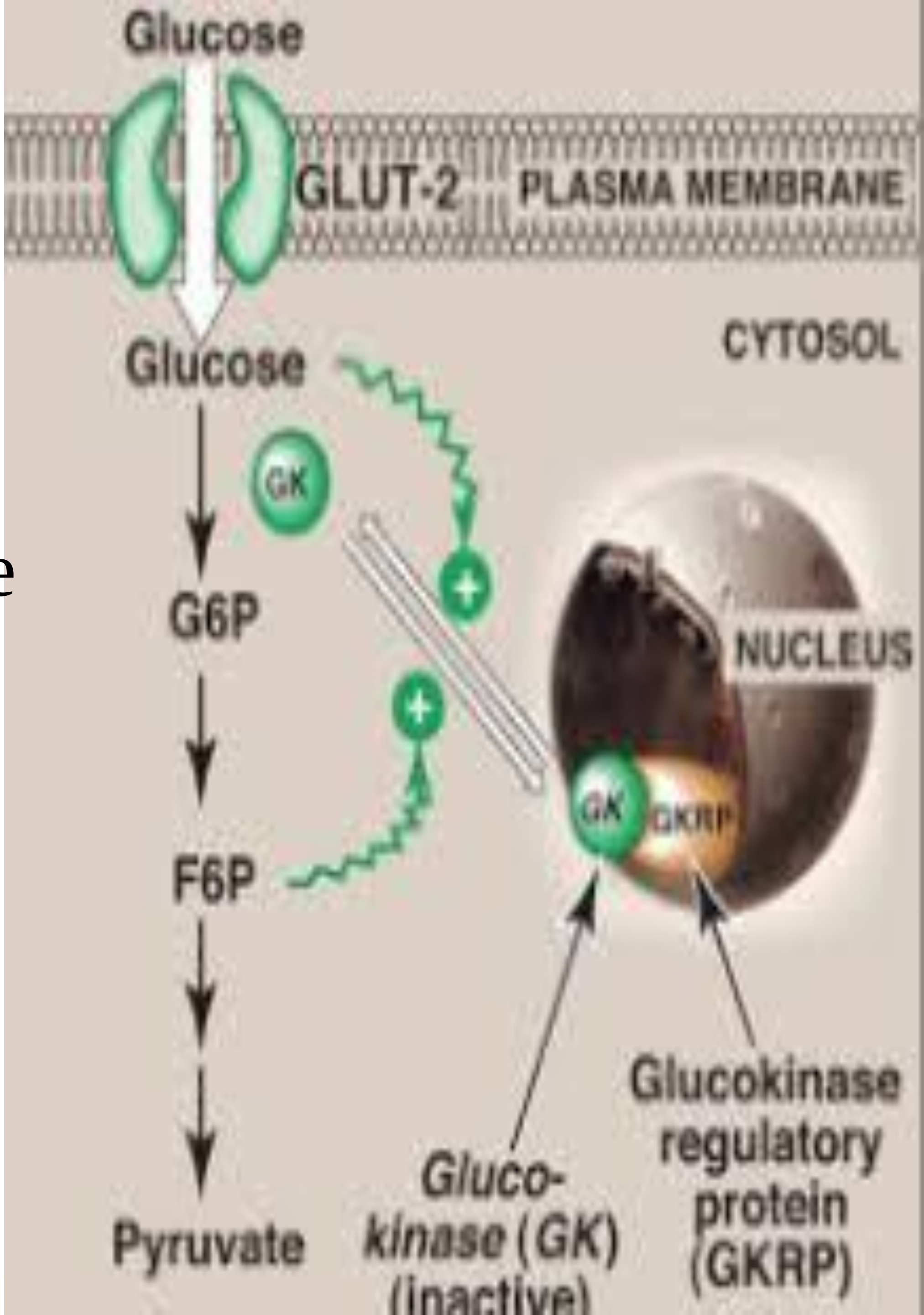
# REGULATION BY GLUCOSE 6 PHOSPHATE & GLUCOSE

- Hexokinase  inhibited by glucose-6 p(product inhibition).

- Glucokinase inducible enzyme ,Glucose through involvement of insulin induce it.

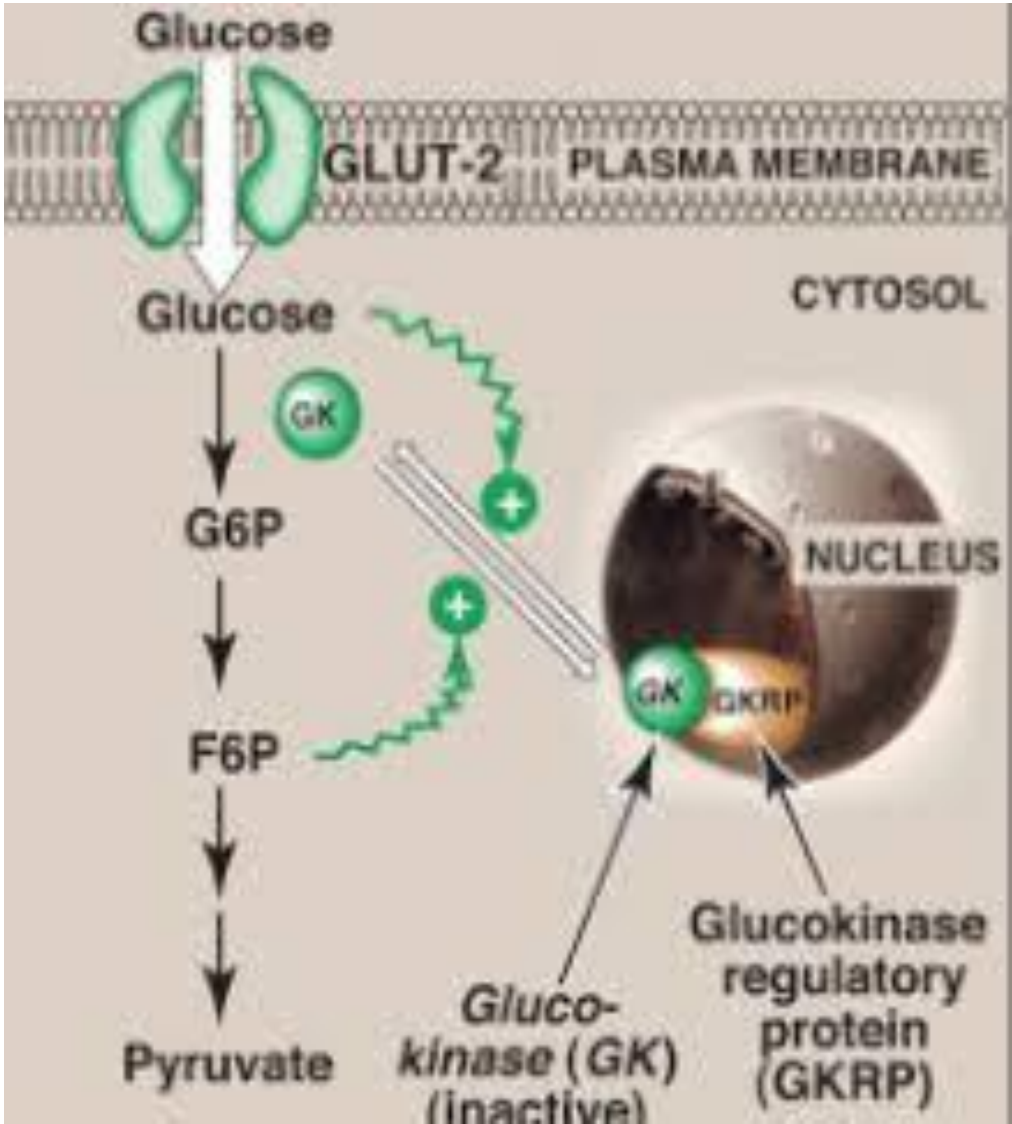
GKRP in the liver regulates the activity of glucokinase through reversible binding.

In the presence of fructose 6 phosphate, glucokinase is translocated into the nucleus and binds tightly to the regulatory protien.and making the enzyme inactive.

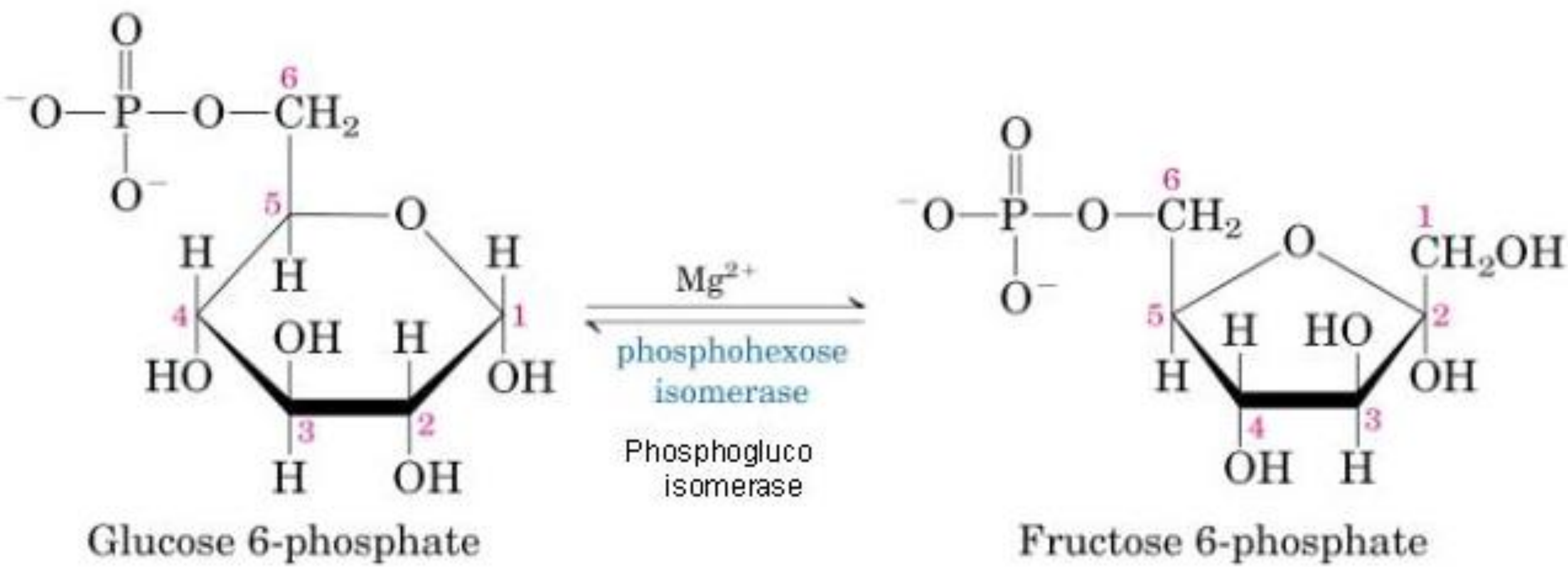


# REGULATION BY FRUCTOSE 6 PHOSPHATE & GLUCOSE

- When the glucose level in the blood rises, glucokinase is released from GKRP and the enzyme re-enters the cytosol where it phosphorylates glucose to glucose 6 phosphate.

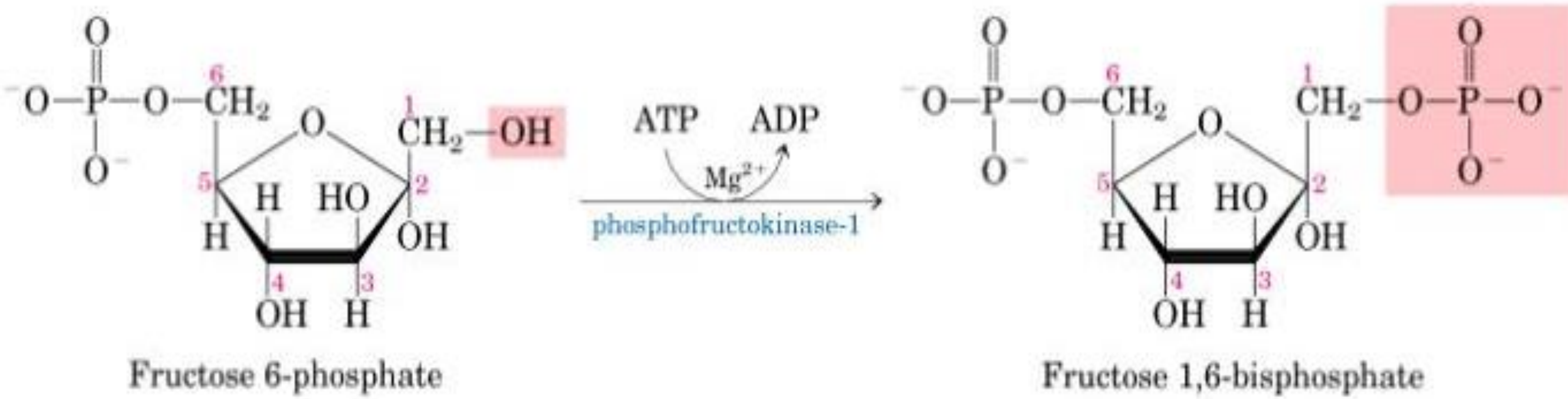


# 2- Isomerization of glucose-6-phosphate
# Enzyme = ***phosphoglucoisomerase***




aldose to ketose isomerization
reversible

# 3- A second phosphorylation.
# Enzyme = ***phosphofructokinase***



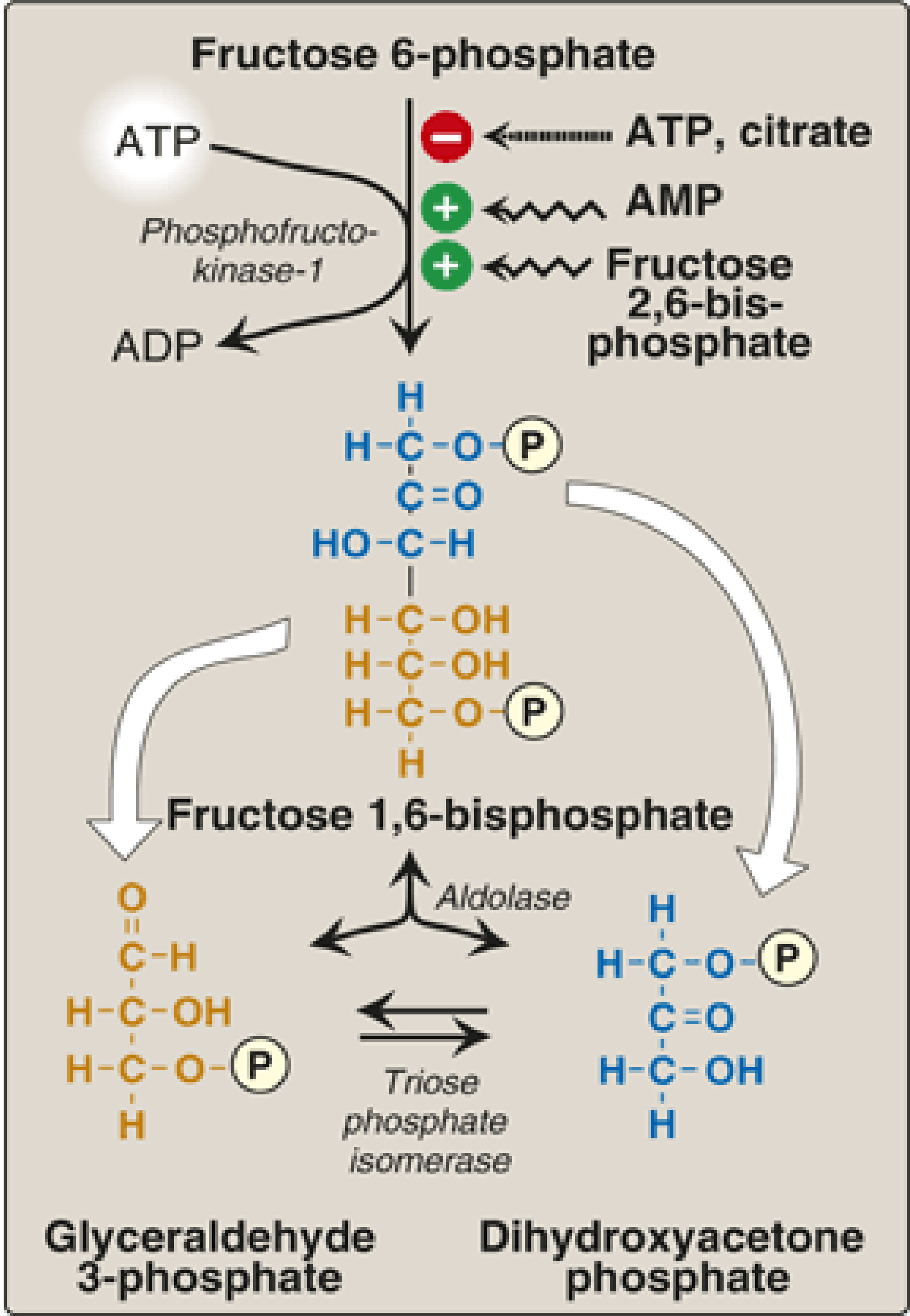

- second ATP investment
- highly exergonic,
- Essentially i*rreversible*,

# Allosteric modification

- Phosphofructokinase-1 is the key regulatory enzyme is subject to feed back inhibition.

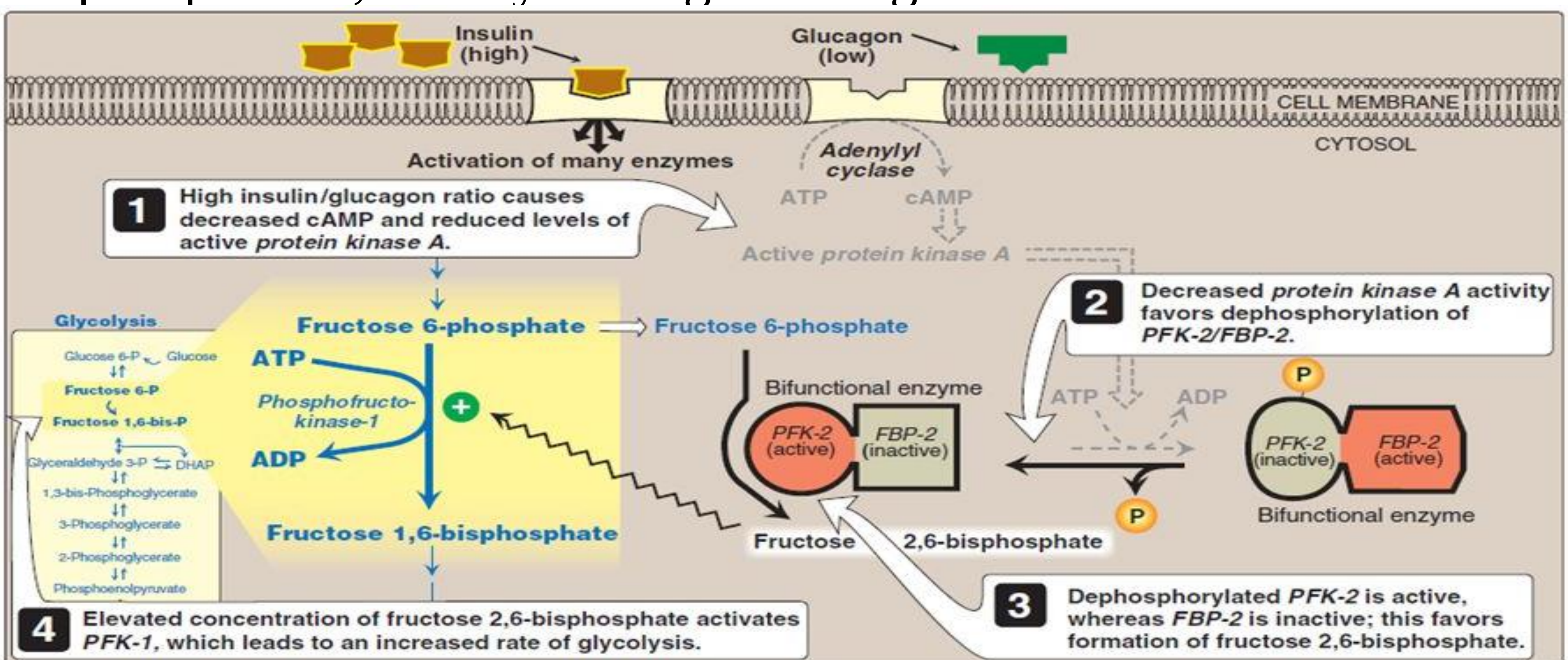
- Enzyme inhibited by citrate and ATP.

- Activated by cAMP.
- Phosphofructokinase -II is an isozyme, catalyze formation of F-2-6 bisphosphate.



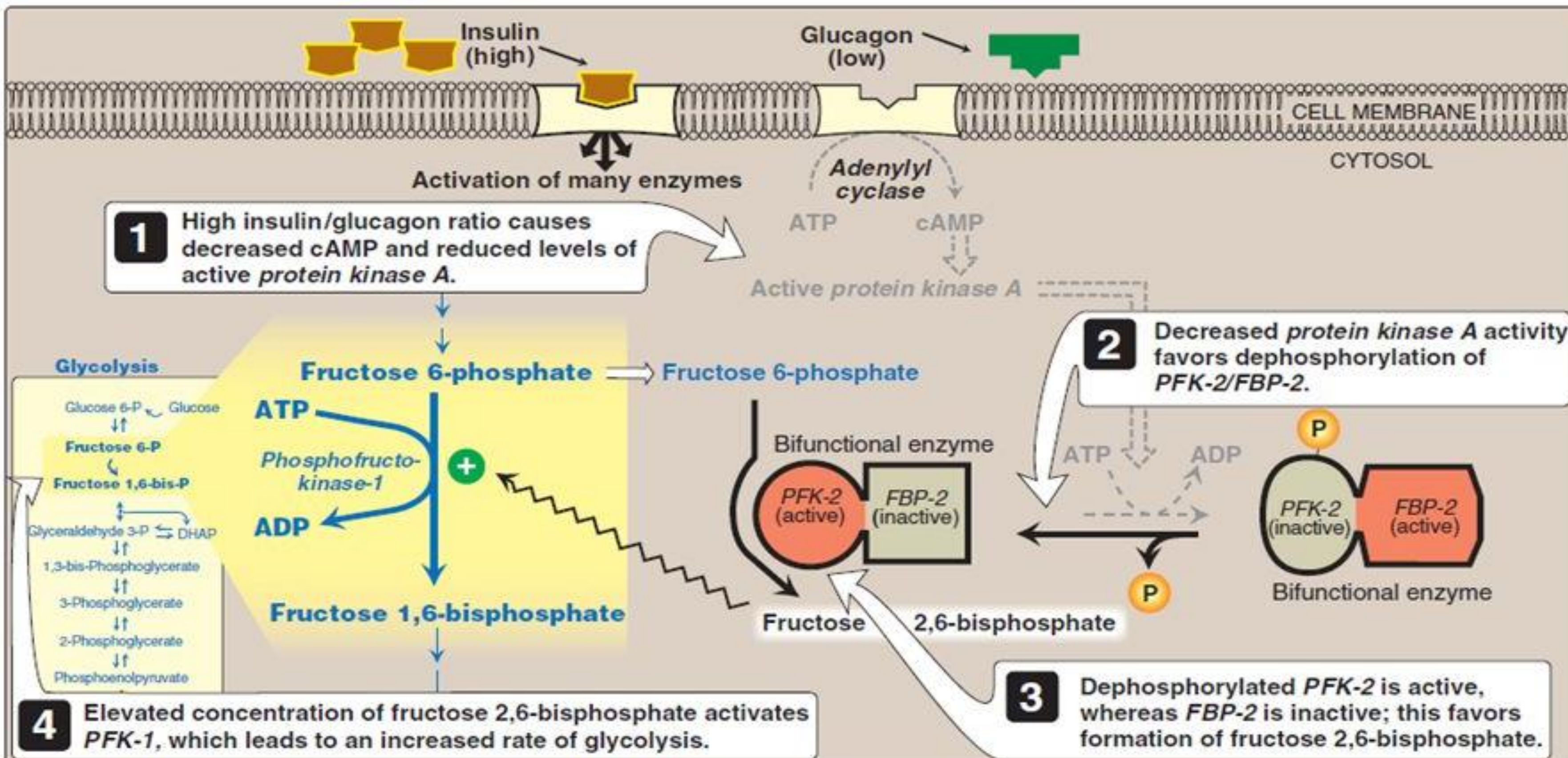
# REGULATION OF PFK1 BY FRUCTOSE 2-6BIS PHOSPHATASE

- Fructose 2-6 bis phosphatase is able to activate PFK1 even when the energy levels are high.
- PFK-II is bifunctional enzyme when phosphorylated, kinase part becomes inactive & no synthesis of fructose 2-6 bis phosphate.
- Fructose 2-6 bis phosphate is inhibitor of fructose 1-6 bis phosphatase,an enzyme of gluconeogenesis.



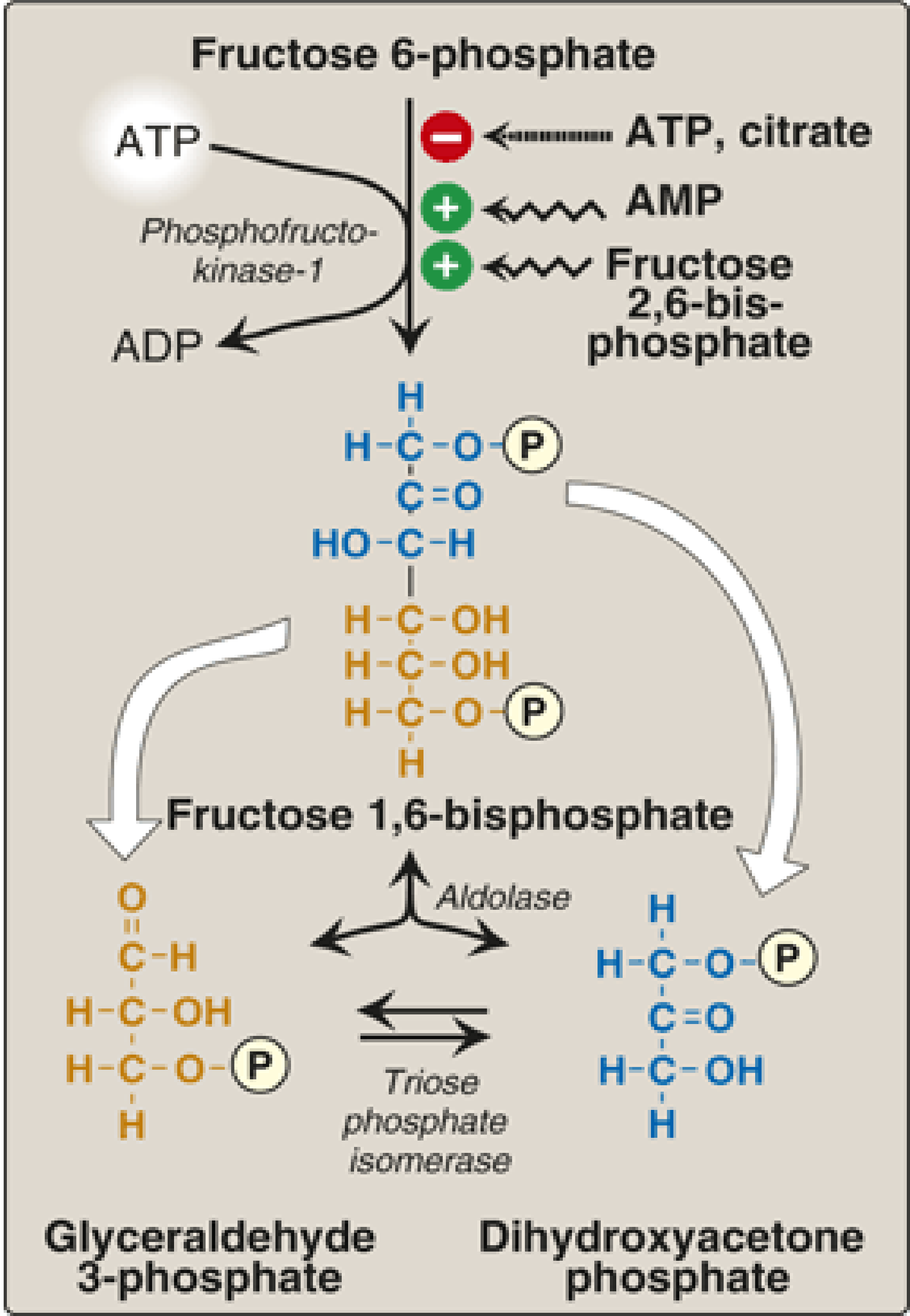
# REGULATION OF PFK1 BY FRUCTOSE 2-6BIS PHOSPHATASE

- There is reciprocal actions of fructose 2-6 bis phosphate on glycolysis(activation) and gluconeogenesis(inhibition),ensures that both the pathways are not fully active at the same time.



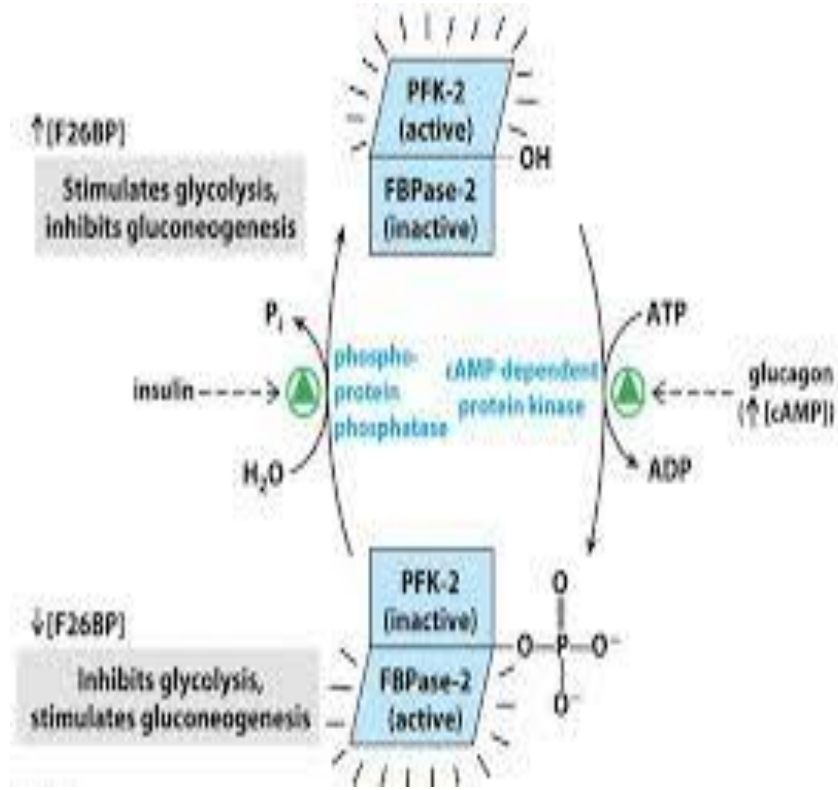
# DURING WELL-FED STATE:

- Decreased level of glucagon and elevated level of insulin , such as occurring after carbohydrates rich meal, cause an increase in fructose 2-6 bis phosphate and thus in the rate of glycolysis in the liver.
- Fructose 2-6 bis phosphate therefore act as intracellular signal, indicating that glucose is abundant.



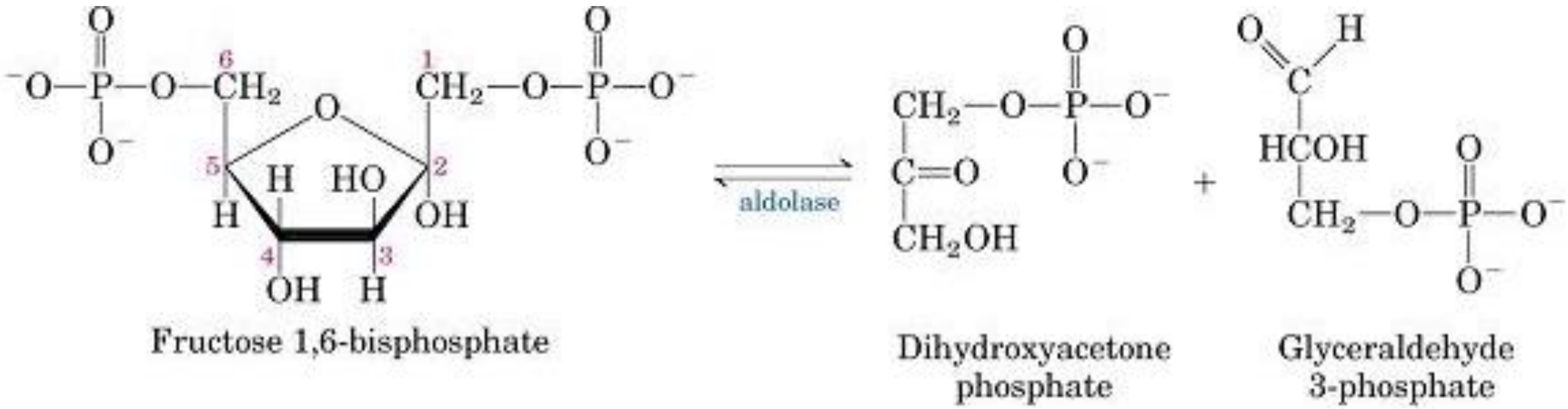
# DURING FASTING:

- Elevated levels of glucagon and low levels of insulin , such as occur during fasting decreases intracellular concentration of hepatic fructose 2-6bis phosphate.
- This results in inhibition of glycolysis and activation of gluconeogenesis.



# SPLITTING PHASE

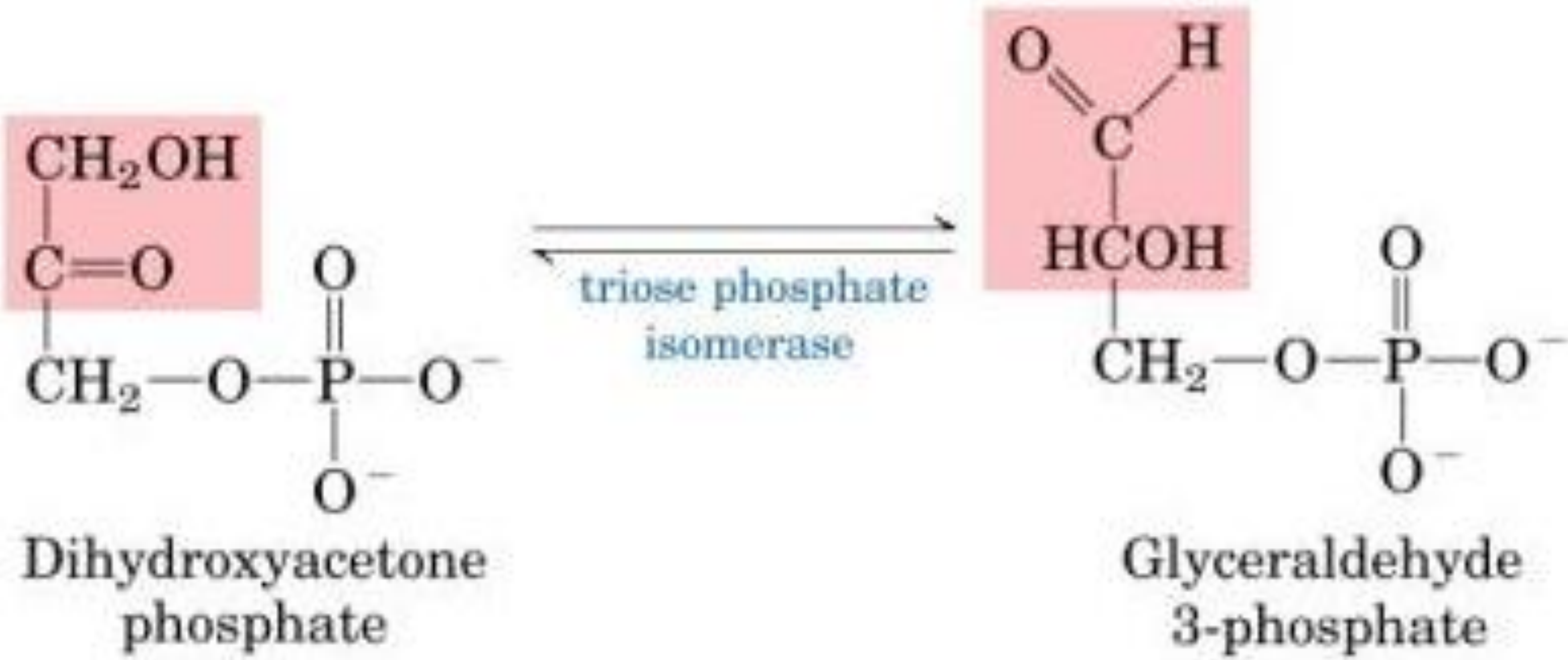
# 4- Cleavage to two triose phosphates Enzyme = ***aldolase***




P453 MVA

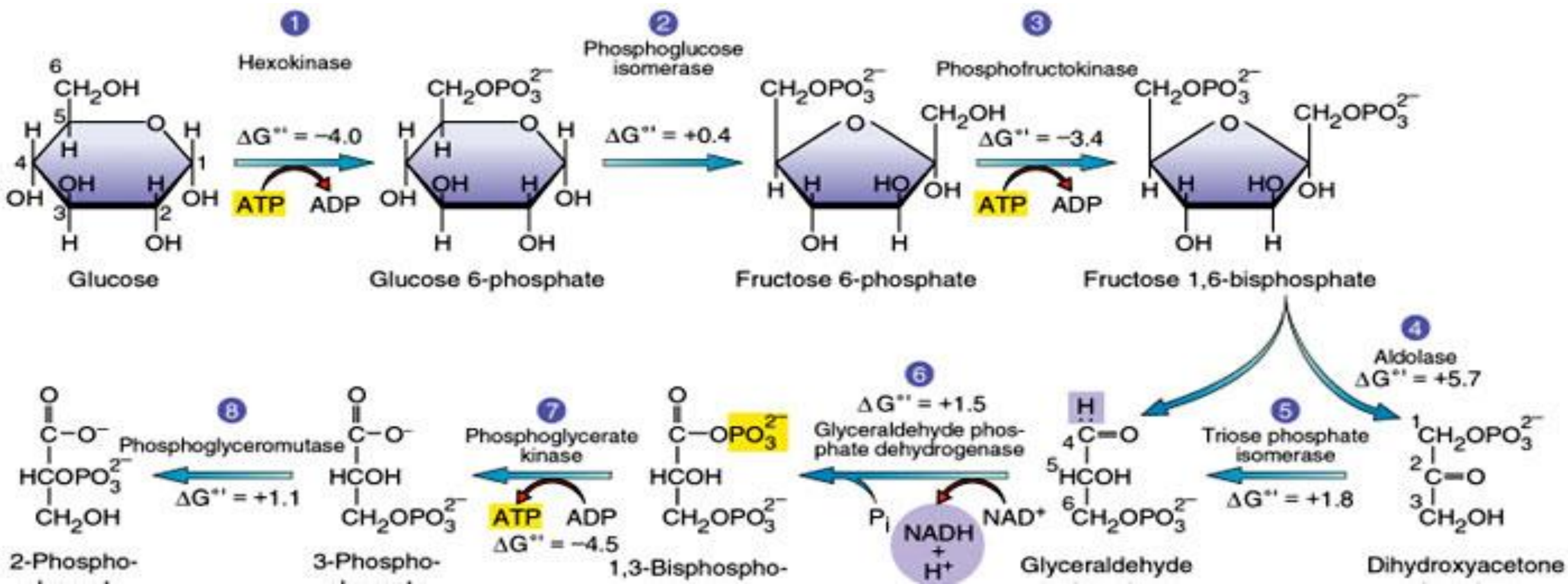
- cleaves a ***6C sugar to two 3C sugars***

# 5- Isomerization of dihydroxyacetone phosphate
# Enzyme = ***triose-phosphate isomerase***



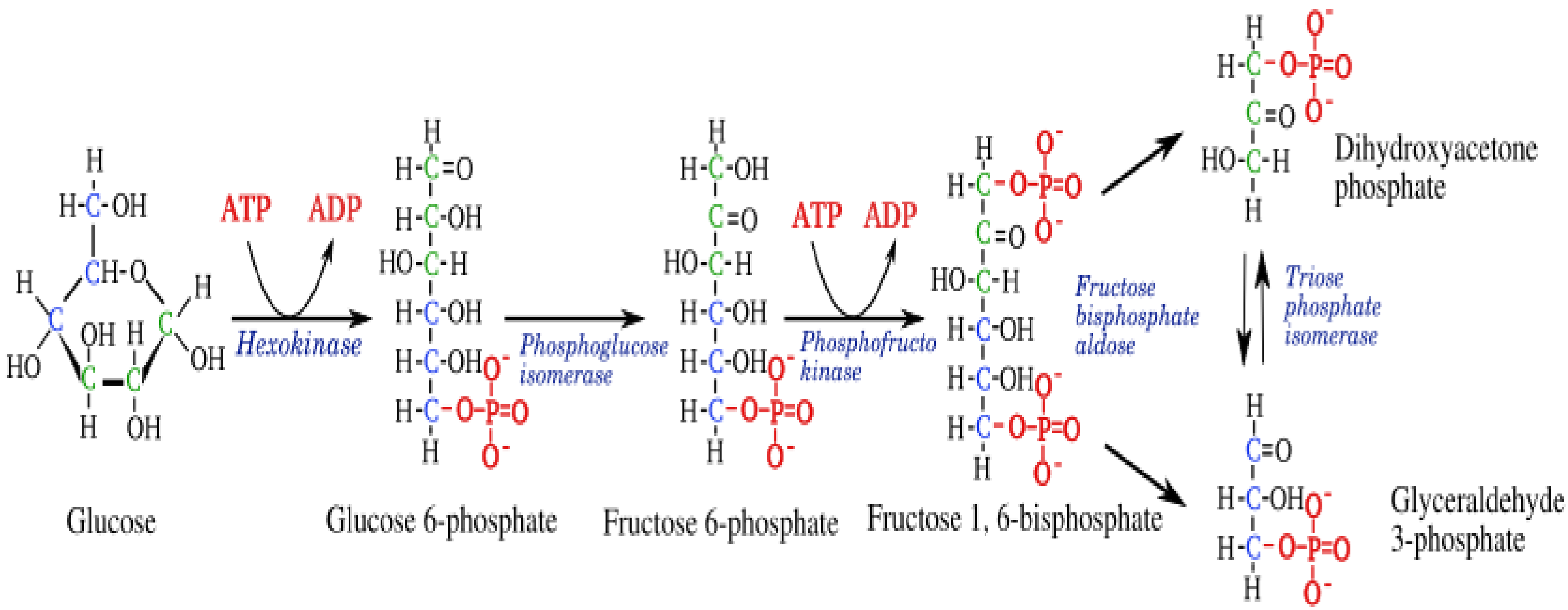
# ISOMERIZATION OF DIHYDROXYACETONE PHOSPHATE

- TRIOSE PHOSPHATE ISOMERAZE interconverts DHAP and glyceraldehyde 3 phosphate.
- DHAP must be isomerized to glyceraldehyde 3 phosphate for further metabolism by glycolytic pathway.



# ISOMERIZATION OF DIHYDROXYACETONE PHOSPHATE

- This isomerization results in the net production of two molecules of glyceraldehyde 3 phosphate from the cleavage products of fructose 1-6 bis phosphate.



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# End of First Phase:

- Production of two glyceraldehyde3-phosphate molecules from one glucose molecule with the expenditure of two ATPs.
- Therefore: the energy yields of the 2nd phase steps are multiplied by two.

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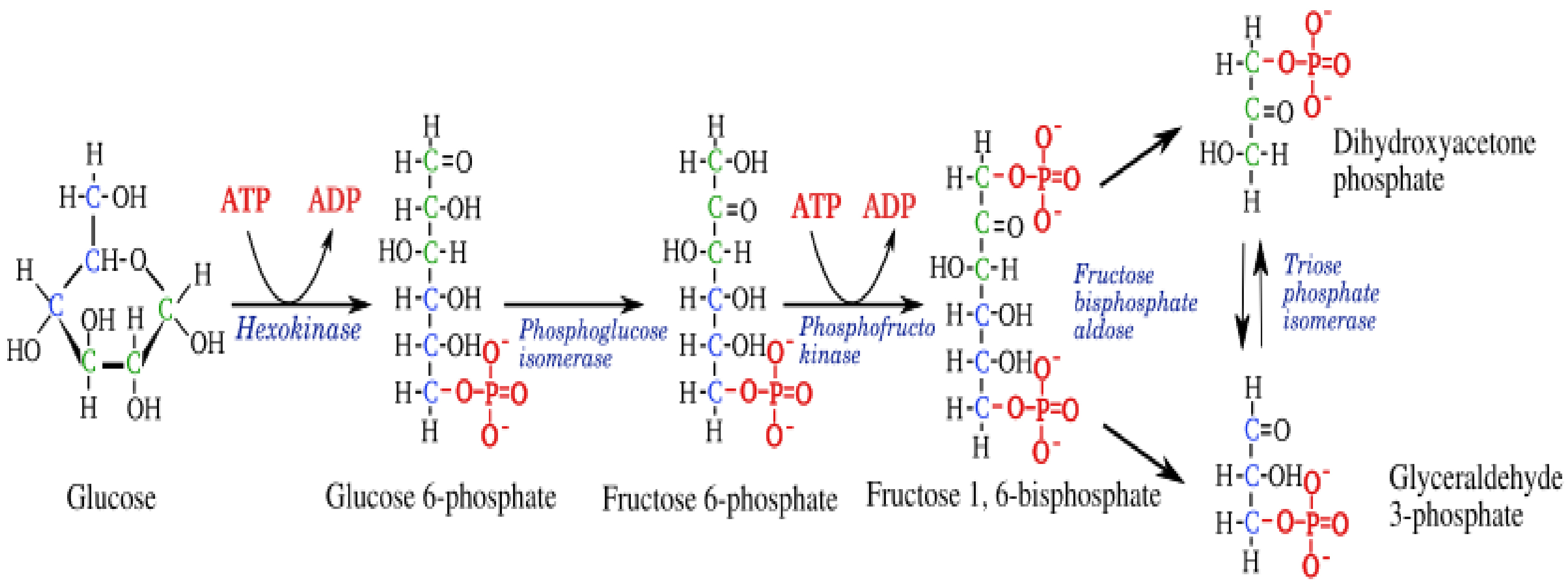
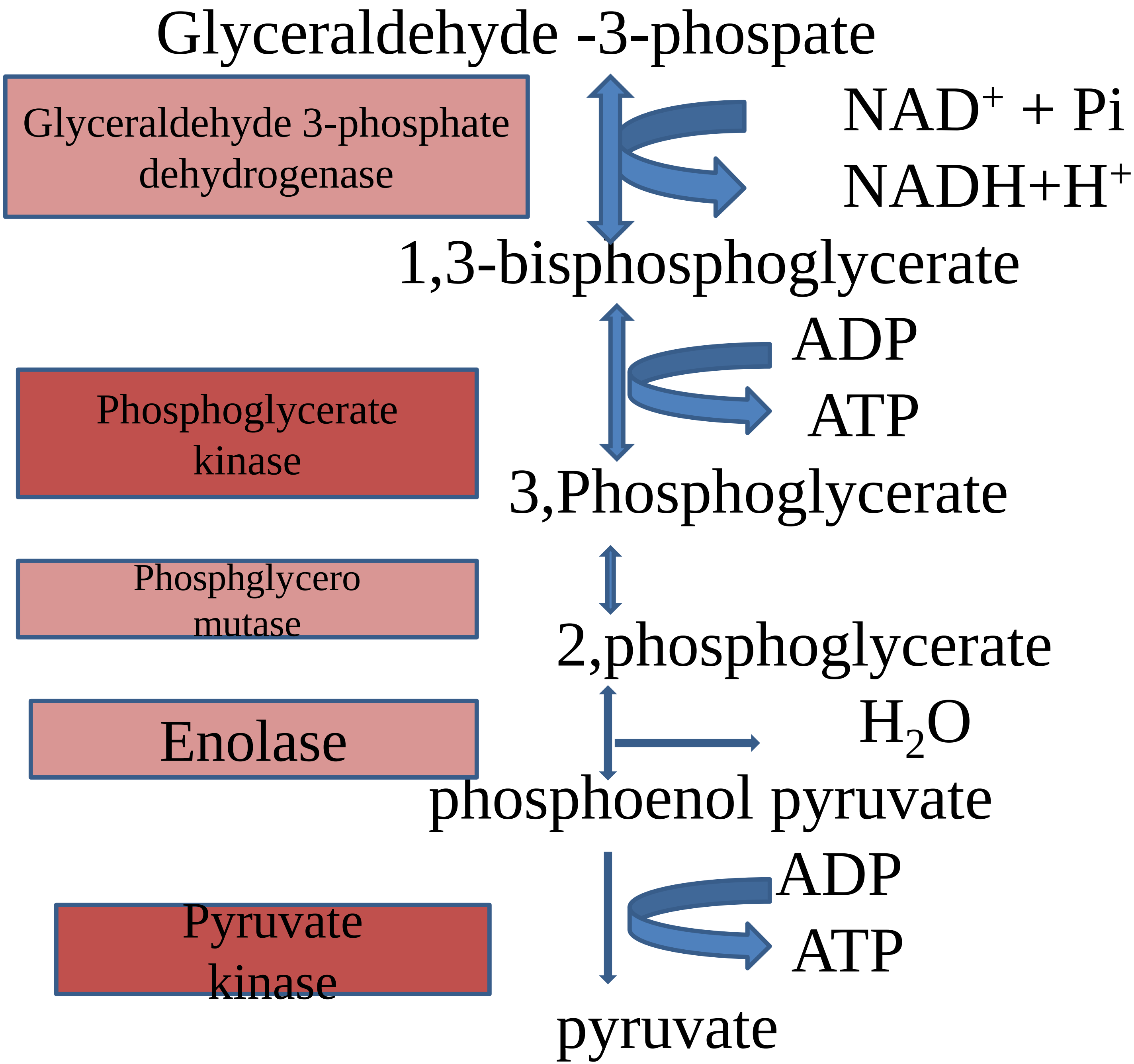


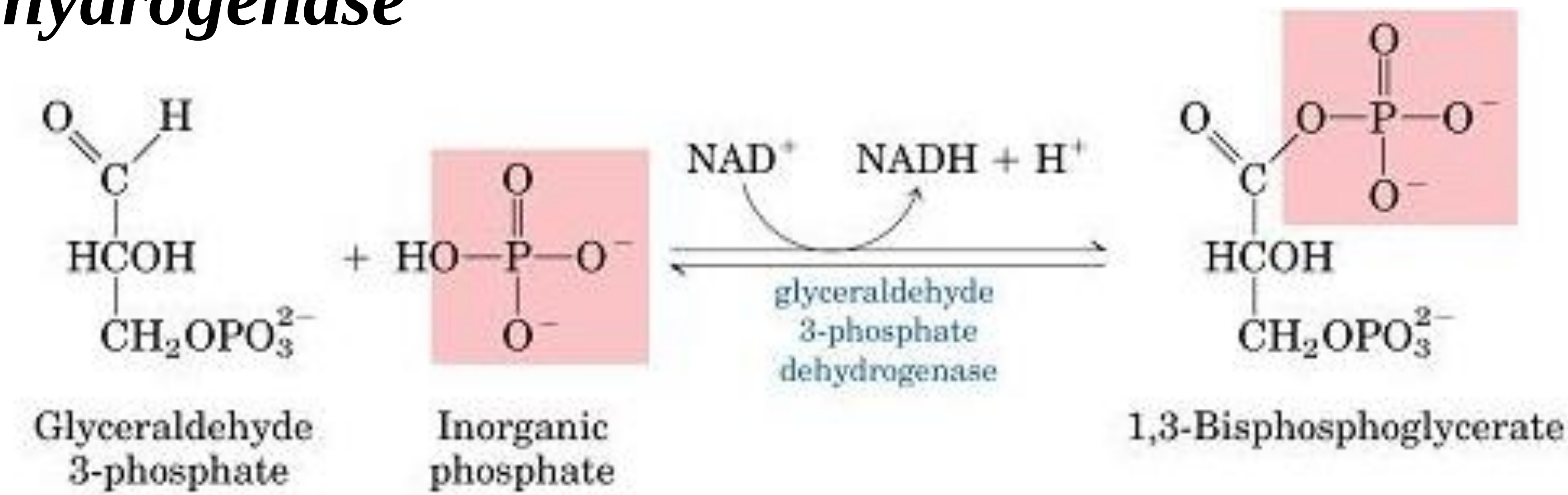
# Second Phase:

Energy generation phase


Glyceraldehyde -3-phospate
Glyceraldehyde 3-phosphate dehydrogenase
$NAD^+$ + Pi
$NADH+H^+$
1,3-bisphosphoglycerate
Phosphoglycerate kinase
ADP
ATP
3,Phosphoglycerate
Phosphglycero mutase
2,phosphoglycerate
Enolase
$H_2O$
phosphoenol pyruvate
Pyruvate kinase
ADP
ATP
pyruvate

6- Oxidation of glyceraldehyde 3-phosphate

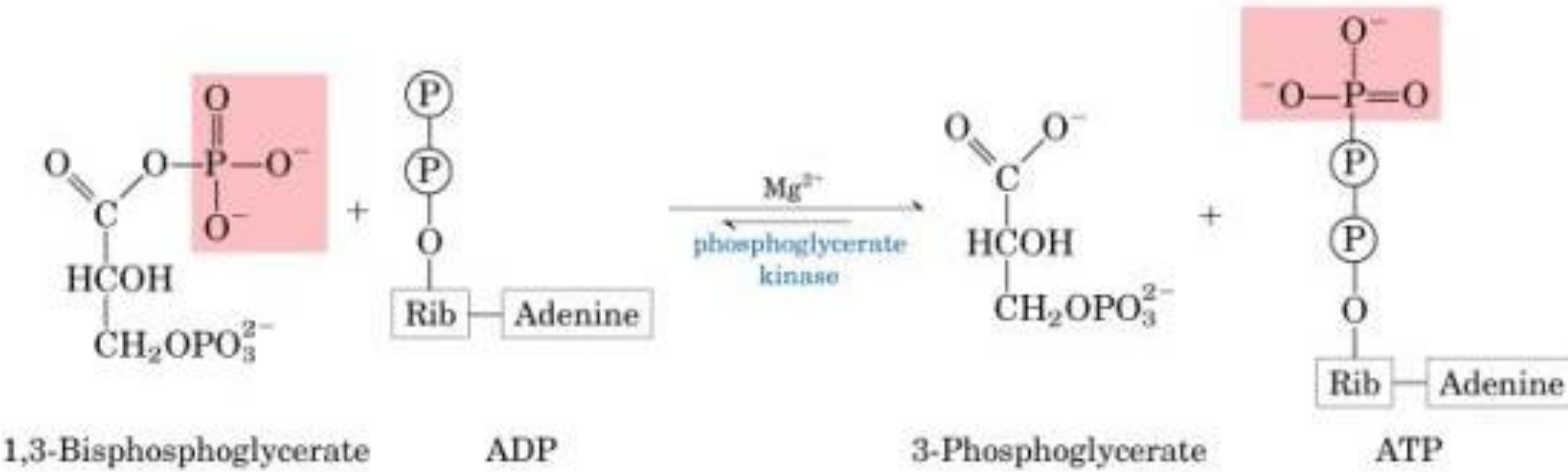
Enzyme = ***glyceraldehyde-3-phosphate dehydrogenase***




- addition of phosphate, oxidation, production of NADH, formation of high energy compound

7- Transfer of phosphate to **make ATP**

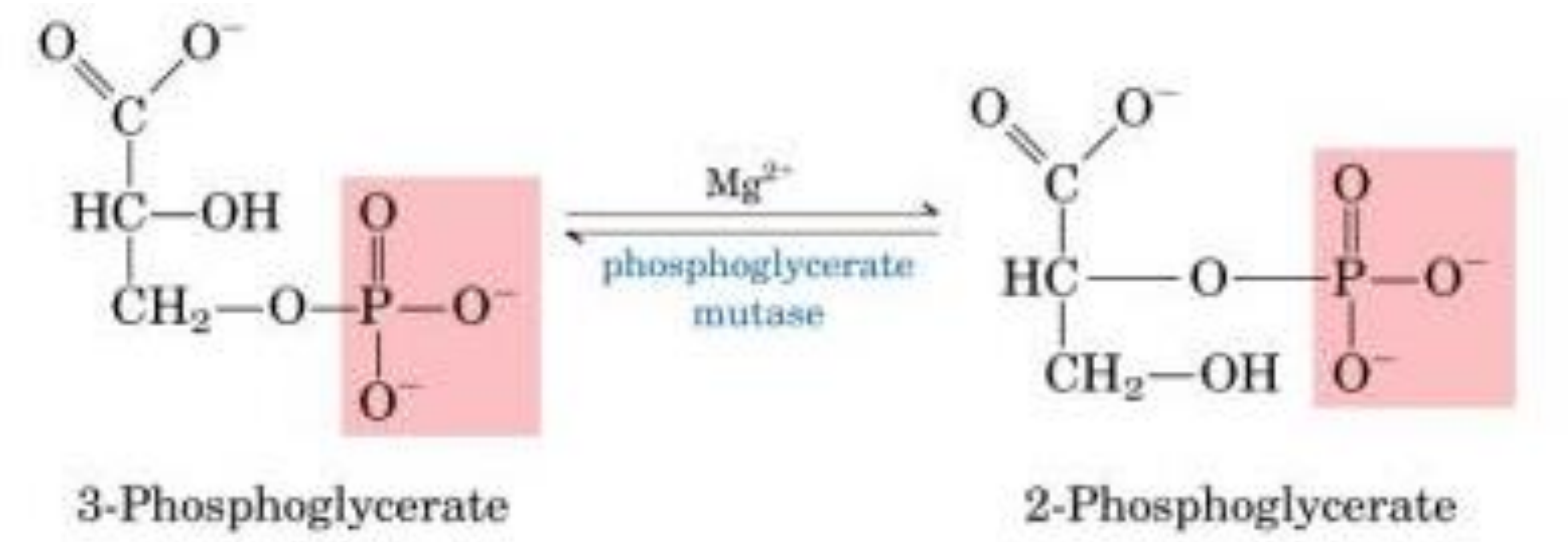
Enzyme = ***phosphoglycerate kinase***




- **first substrate level phosphorylation, yielding ATP**
- **two 1-3 bis PG yield 2 ATPs**

# 8- Phosphate shift setup

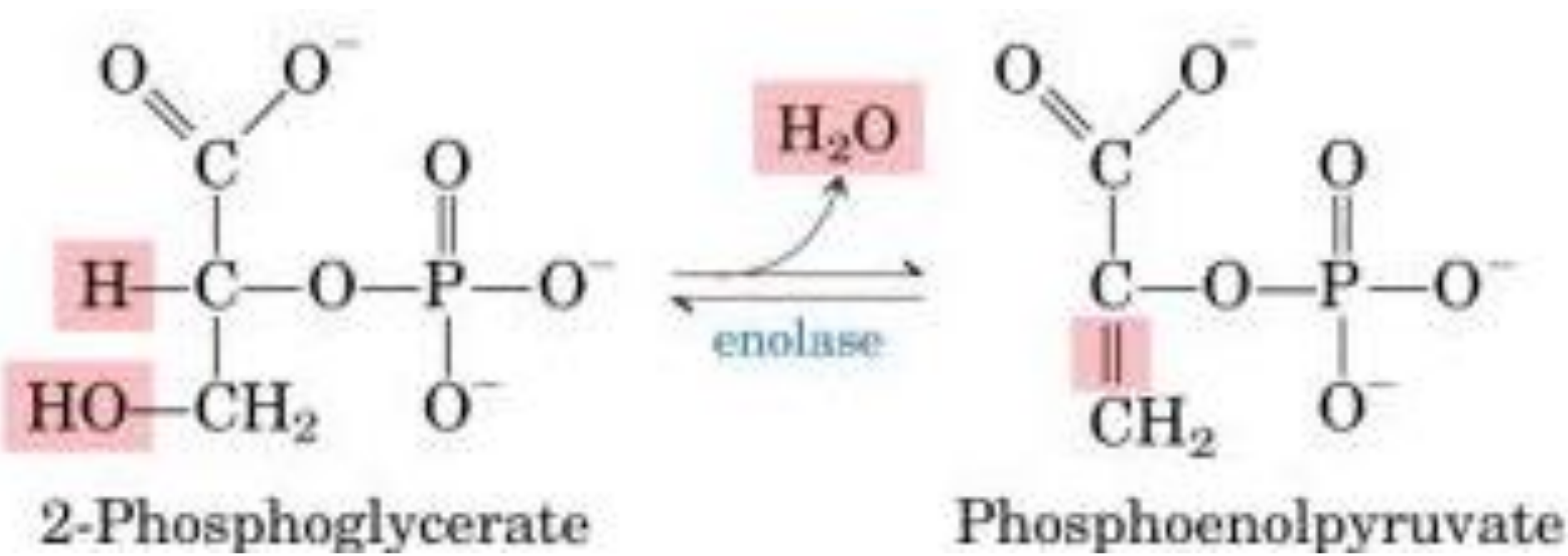
Enzyme = ***phosphoglycerate mutase***




- transfer phosphate from position 3 to 2.
- *reversible*

# 9- Generation of second very high energy compound by a ***dehydration***

Enzyme = ***enolase***



10- Final generation of ATP

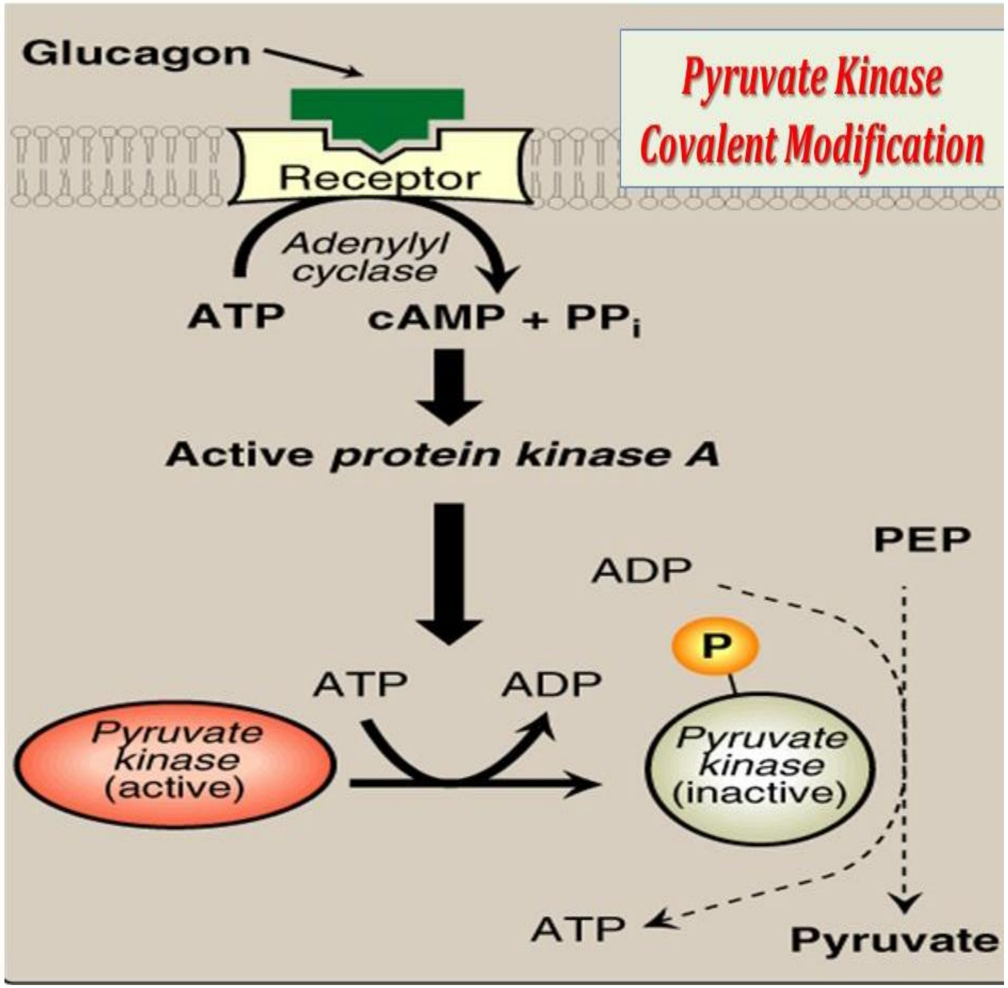
Enzyme = ***pyruvate kinase***


*phosphoenolpyruvate* *pyruvate*

- second substrate level phosphorylation yielding ATP
- highly exergonic reaction, *irreversible*

# COVALENT MODULATION OF PYRUVATE KINASE:

Harmones like glucagon and epinephrine activate cAMP dependant protein kinase which can phosphorylate and inactivate key enzyme pyruvate kinase,and thus inhibit glycolysis.



# Production of ATP in glycolysis

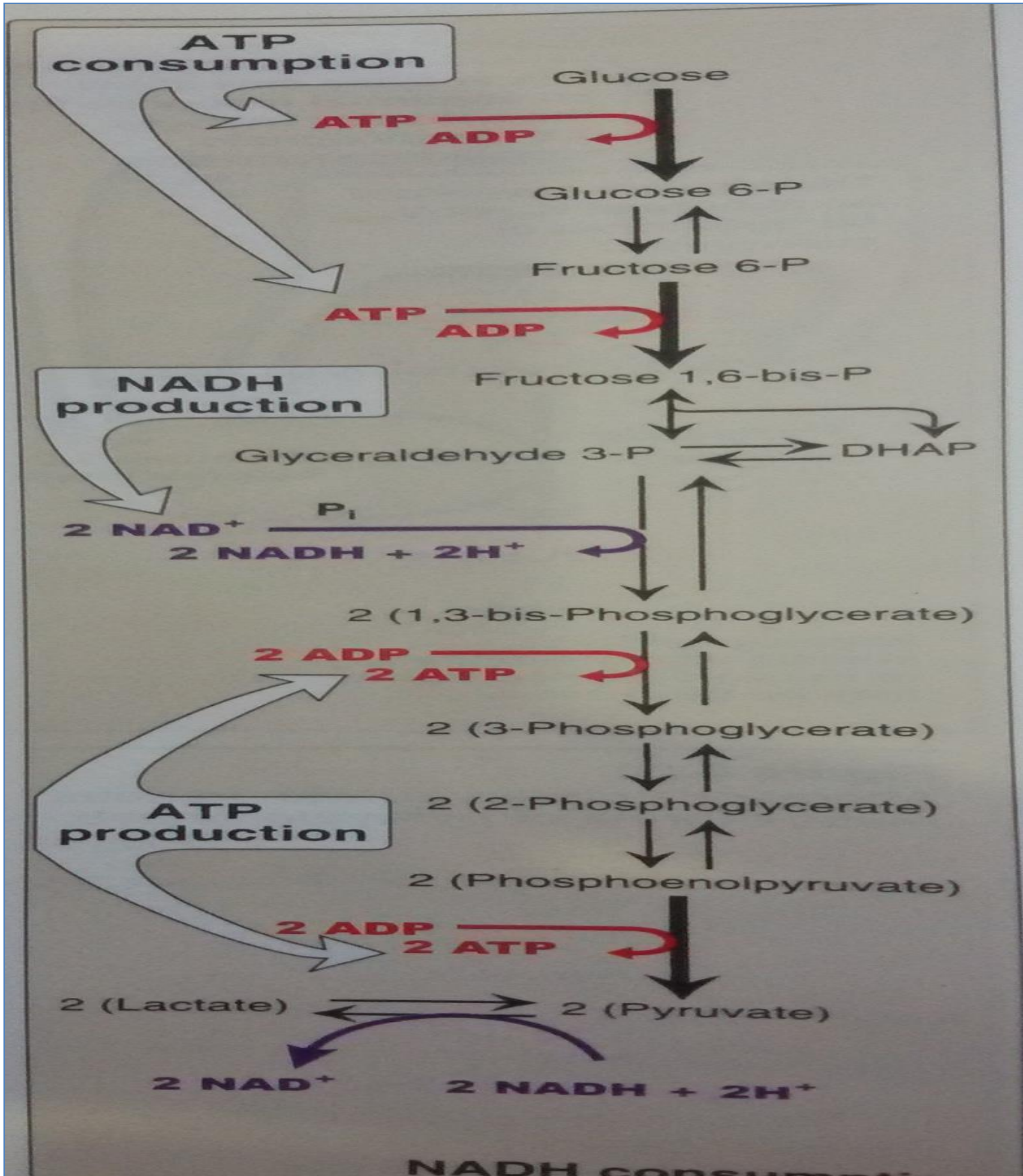
STAGE -1

- Hexokinase /Glucokinase reaction → - 1 ATP
- Phosphofructokinase-1 → - 1 ATP

STAGE -2

- Glyceraldehyde -3-P dehydrogenase
- (oxidation of 2 NADH in electron transport chain),

  + 6 ATP
- Phosphoglycerate kinase
- (substrate level phosphorylation) +2 ATP



ATP consumption
Glucose
ATP
ADP
Glucose 6-P
Fructose 6-P
ATP
ADP
Fructose 1,6-bis-P
NADH production
Glyceraldehyde 3-P
DHAP
Pi
2 NAD+
2 NADH + 2H+
2 (1,3-bis-Phosphoglycerate)
2 ADP
2 ATP
2 (3-Phosphoglycerate)
2 (2-Phosphoglycerate)
ATP production
2 (Phosphoenolpyruvate)
2 ADP
2 ATP
2 (Lactate)
2 (Pyruvate)
2 NAD+
2 NADH + 2H+
NADH consumption

# Continue ATP production

STAGE -3

Pyruvate kinase

(substrate level phosphorylation) + 2 ATP

Net gain =10-2

=8ATP

- In anaerobic glycolysis reoxidation of NADH at glyceraldehyde-3-P-dehydrogenase can not take place in ETC.
- So ATP production per molecule of glucose oxidation is 4- 2=2 ATP.

# Mode of regulation

- Level of substrate

Enzymes

Level of Energy

Level of enzymes cofactors

# Regulation of glycolysis

Induction/repression

Increase in substrate ie glucose activate enzymes involved in utilization.

Enzymes producing glucose(gluconeogenesis) are inhibited.

Insulin enhances key enzymes for glycolysis , inhibit enzymes of gluconeogenesis.

# Regulation of glycolysis

- Three enzymes
- Hexo kinase/Glucokinase
- Phospho fructokinase
- Pyruvate kinase
- Catalyzing irreversible reactions regulate glycolysis.

- To metabolize glucose beyond pyruvate ___ requires
- ***OXYGEN***
- ***MITOCHONDRIAL ENZYME SYSTEM:***

1. ***Pyruvate Dehydrogenase Complex***
2. ***Citric Acid Cycle***
3. ***Respiratory Chain***

# *FATE OF PYRUVATE*

Pyruvate  is the product of glycolysis

Pyruvate is at a ***central branch point*** of metabolism

-***Pyruvate*** can be further processed:

a) *anaerobically* to **lactate** in muscle and in certain micro-organisms via lactate dehydrogenase

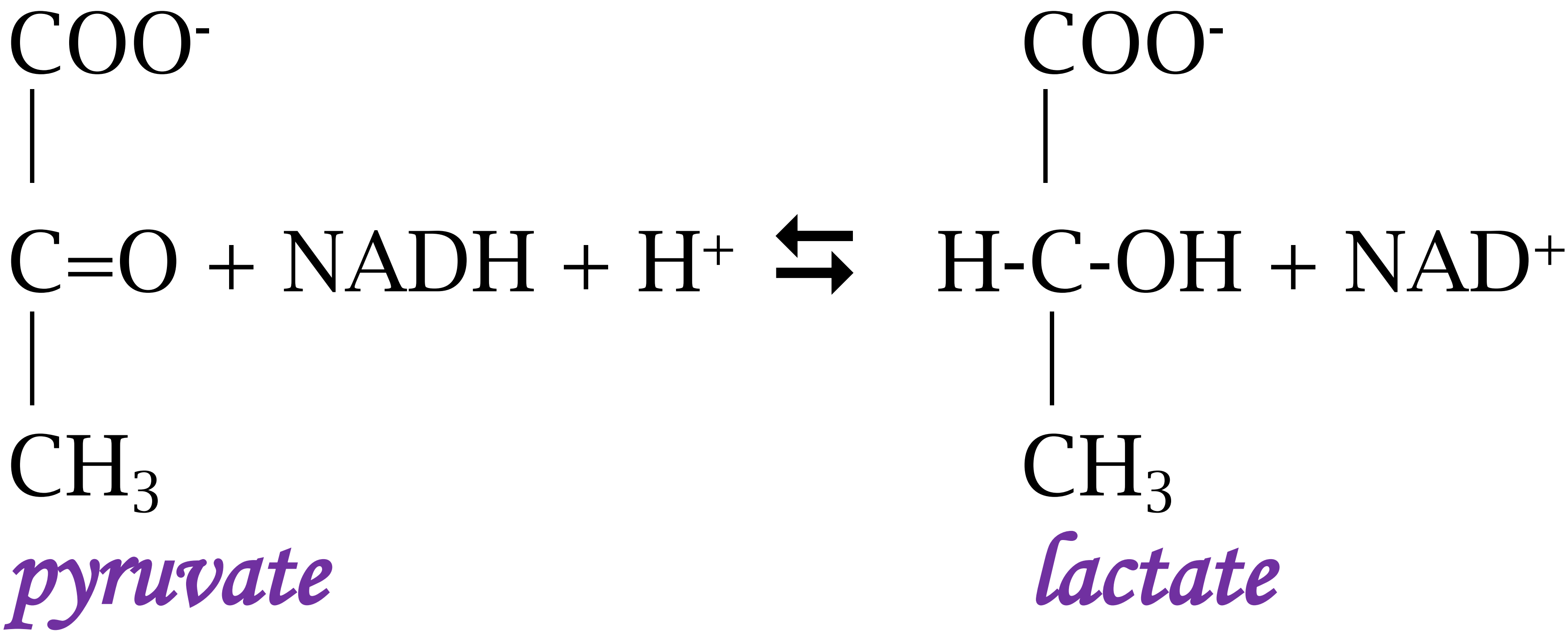
b) *anaerobically* to **ethanol** (**fermentation**) via ethanol dehydrogenase

***or***

c) *aerobically* to $CO_2$ and $H_2O$ via the citric acid cycle.

# Lactate Fermentation

Enzyme = ***Lactate Dehydrogenase***




Note: uses up all the NADH (reducing equivalents) produced in glycolysis.

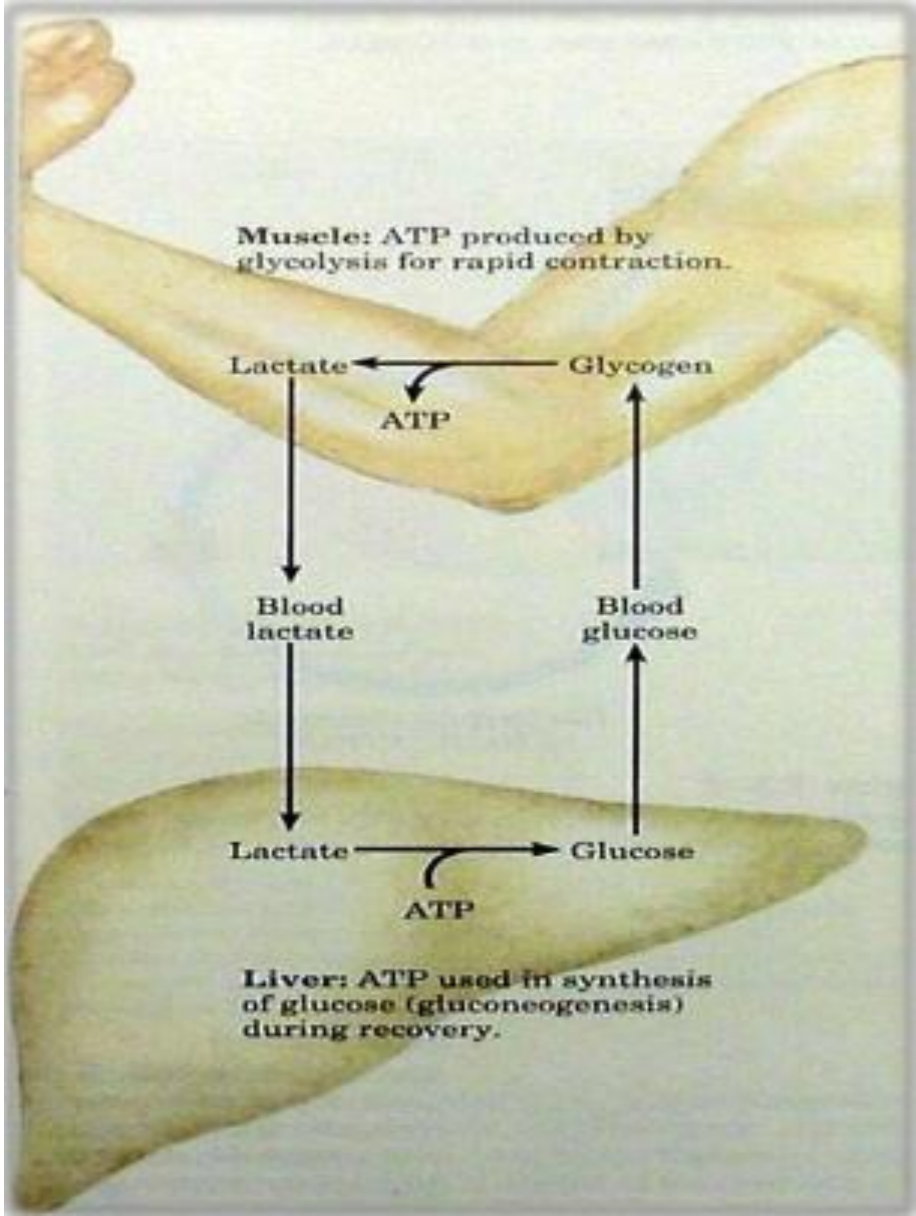
- Helps *drive* glycolysis by using up NADH
- *reversible* so pyruvate can be regenerated in alternative metabolism
- lactate fermentation important in red blood cells, renal medulla, lens of eye and in skeletal *muscle* cells during strenuous exercise. Also important
in *plants* and in *microbes* growing in absence of $O_2$.

# LACTATE UTILIZATION:

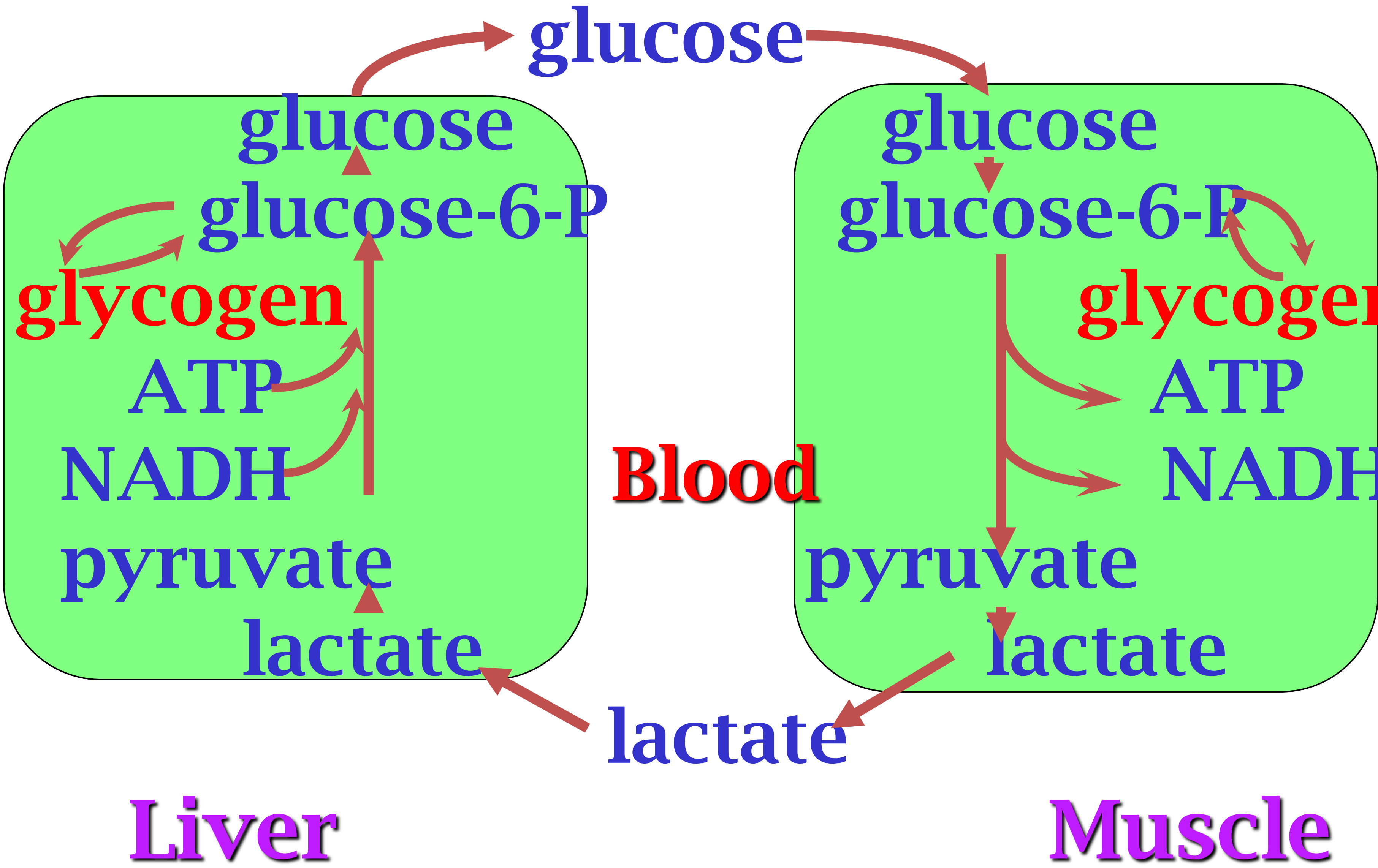
- The direction of the lactate dehydrogenase reaction depends on the relative intracellular concentrations of pyruvate and lactate and the ratio of NADH/NAD in the cell.
- E.g ,in the liver and heart , the ratio of NADH/NAD is lower than in exercising muscle. These tissues oxidize latate to pyruvate.

# LACTATE FORMATION IN MUSCLES:

- During intense exercise lactate accumulates in muscles , causing a drop in intracellular pH , potentially resulting in cramps.
- Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose.



# LACTIC ACID (CORI) CYCLE

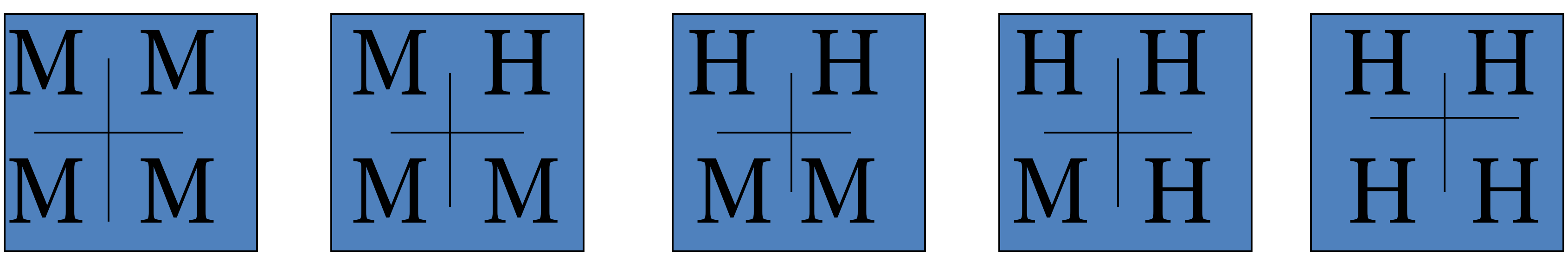


# LACTIC ACIDOSIS:

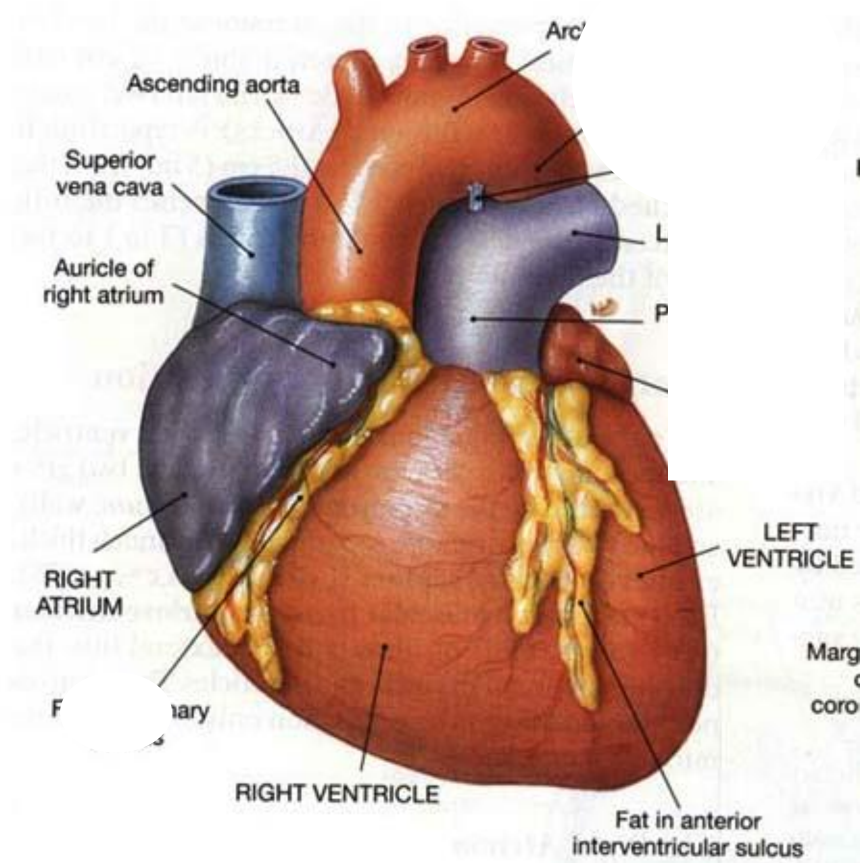
- Elevated concentration of lactate in the plasma.
- Occurs in MI, PULMONARY EMBOLISM , HEMORRHAGE or SHOCK.

-- ***Lactate Dehydrogenase (LDH)*** has multiple forms. It is an isozyme. Two polypeptides M and H come together to form LDH. It is a tetramer so a mixture is formed:

**$M_4$, $M_3H$, $M_2H_2$, $MH_3$ and $H_4$**



- Skeletal muscle and liver contain predominantly the “**M**” forms; heart the “**H**” forms. During and after myocardial infarction, heart cells die releasing LDH into the circulation.




- Diagnostic.

# FATE OF PYRUVATE:

- OXIDATIVE DECARBOXYLATION OF PYRUVATE:

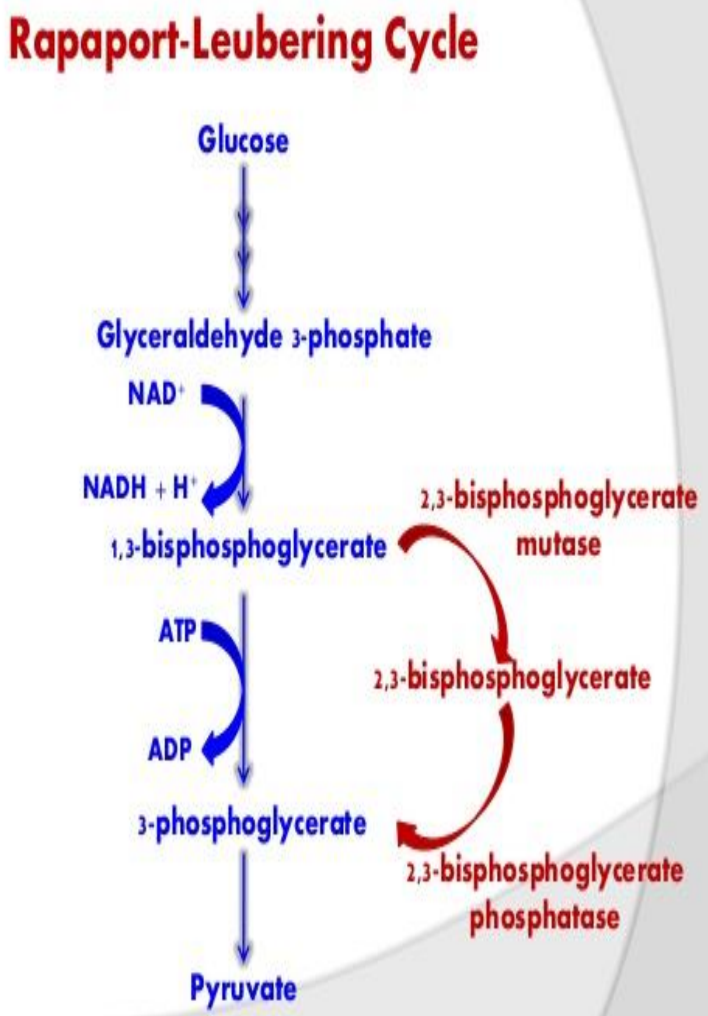
  PDH complex is and important pathway in the tissues having high oxidative capacity ,such as cardiac muscles.
- PDH complex irreversibly converts pyruvate (the and product of glycolysis) into acetyl COA(major fuel of TCA cycle).

# B) CARBOXYLATION OF PYRUVATE TO OXALOACETATE:

- Carboxylation of pyruvate to oxaloacete is a biotin dependent reaction.
- This reaction is important because it replenishes the TCA cycle intermediates and provides substrate for gluconeogenesis.
- C) REDUCTION OF PYRUVATE TO ETHANOL.

# Rapaport –leubering cycle

- Glycolysis in erythrocytes is linked with 2,3-Bisphosphoglecerate production and oxygen transport.
- It  combines with Hb and reduces Hb affinity for oxygen, so oxy hemoglobin unloads more oxygen to tissues.




Thank you!