BIOENERGETICS AND METABOLISM

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Biochemistry II

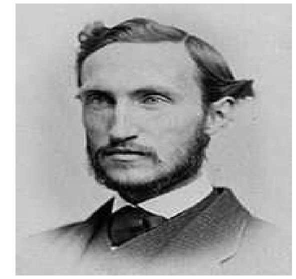
Learning Objectives

- Define free energy & predictions of spontaneity
- Describe the characteristics of metabolism
- Discuss ATP as energy currency
- Describe the uses, principles & regulation of metabolism
- Describe how carbohydrate metabolized by cells
- Lactate metabolism & Cori cycle
- Regulation of carbohydrate metabolism
- Entry of other monosaccharide to glycolytic pathway

BIOENERGETICS

- All living organisms have the ability to produce energy and to channel it into biological work
- Living organisms carry out energy transductions, conversions of one form of energy to another form
- Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical process underlying these transductions

Free Energy Changes in Metabolic Reactions



1839 – 1903

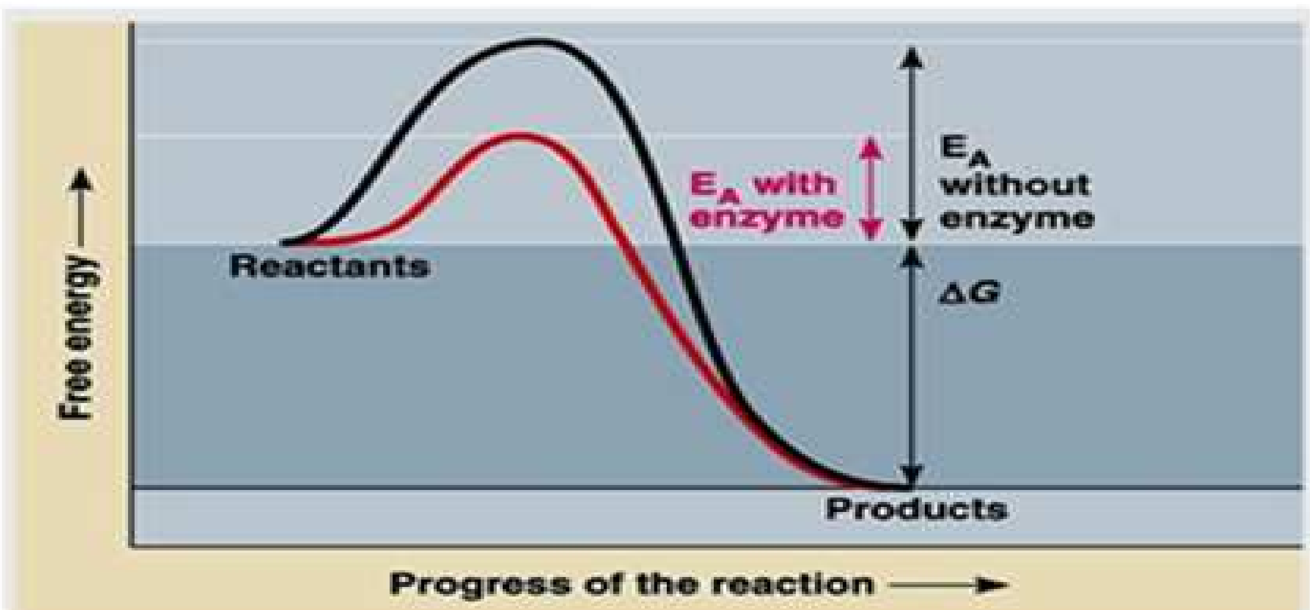
- The energy relevant to biochemical systems is called the Gibbs free energy.
- J W Gibbs (1873) combined 1st and 2nd Laws of thermodynamics to express spontaneity of reactions in terms of measurable system parameters

$$\Delta G = \Delta H - T\Delta S$$

H = Enthalpy (J/mol)
S = Entropy (J/k.mol)
T= Temperature (K)
G= Gibbs free energy (J/mol)

Enzymes and Gibbs free energy

- It is important to remember that enzymes do not change whether a reaction is exergonic or endergonic. This is because they do not change the free energy (Δ G) of the reactants or products.
- Enzymes only reduce the activation energy required for the reaction to go forward.



Free Energy and K_{eq}

- When a reaction is at equilibrium, the concentrations reactants define the equilibrium constant, K_{eq}:

$$A + B \rightleftharpoons C + D \qquad\qquad K_{eq} = \frac{[C][D]}{[A][B]}$$

- When a reaction is not at equilibrium, the reactants experience a driving force to reach their equilibrium values. This force is the free energy.

- In a living cell, reactants and products are almost never present at standard state concentrations.
- Thus, it is important to distinguish the standard free energy change (ΔG^o) of a reaction from its actual free energy change (ΔG).
- ΔG is related to the ΔG^o change for the reaction:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]}$$

Biochemical Standard State

Temperature	25°C (298 K)
Pressure	1 atm
Reactant concentration	1 M
pH	7.0 ($[H^+] = 10^{-7}$ M)
Water concentration	55.5 M

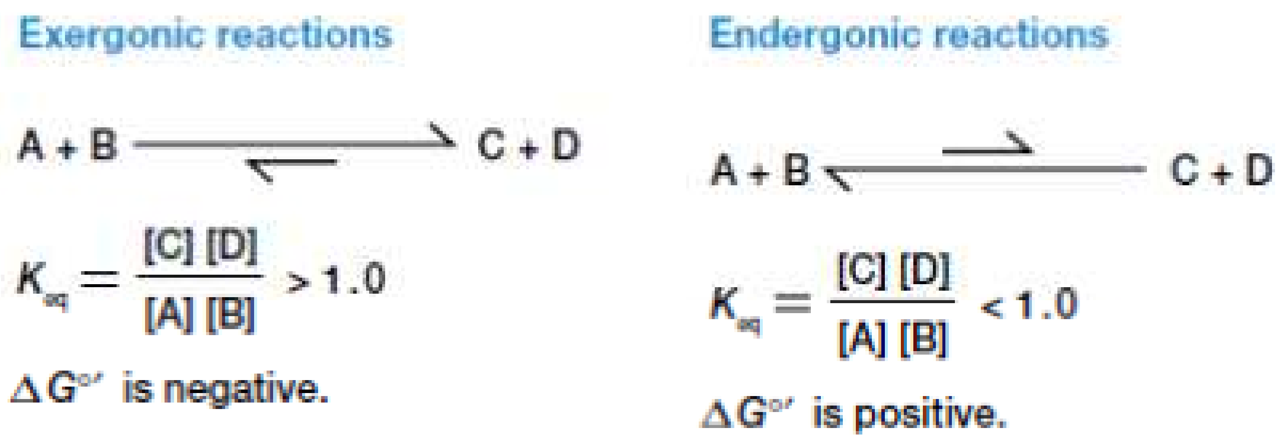
R = 8.3145 J. K^{-1} mol^{-1}, the gas constant.

T = 298 K; to express T in K, add 273 to T in ^oC

- When the reaction is at equilibrium, ΔG = 0

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]}$$

$$\Delta G^{\circ\prime} = -RT \ln \frac{[C][D]}{[A][B]}$$



- Spontaneous (Favorable) reactions have ΔG < 0 (Exergonic)
- Non-spontaneous (Unfavorable) reactions have ΔG > 0 (Endergonic).
- ΔG = 0 the reaction is at equilibrium

- The free energy of the forward reaction (A→B) is equal in magnitude but opposite in sign to backward reaction (B→A).
- Example, if ΔG of the forward reaction is−5 kcal/mol, then that of the back reaction is +5 kcal/mol.
- Standard free energy changes are additive.

$$A \longrightarrow B \qquad \Delta G'^{0}_{1}$$

$$B \longrightarrow C \qquad \Delta G'^{0}_{2}$$

$$\text{Sum:} \quad A \longrightarrow C \qquad \Delta G'^{0}_{1} + \Delta G'^{0}_{2}$$

- Endergonic reaction can be driven in the forward direction by coupling it to a <u>highly exergonic reaction</u>

$Glucose + P_i \longrightarrow G\text{-}6\text{-}P + H_2O$ ($\Delta G'^0$ =13,8 kj/mol)

$ATP + H_2O \longrightarrow ADP + P_i$ ($\Delta G'^0$ = -30,5 kj/mol)

Sum: Glucose + ATP $\longrightarrow$ G-6-P + ADP

- The overall standard free energy changes:

$\Delta G'^0$ =13,8 kj/mol + (-30,5 kj/mol) = -16,7 kj/mol (Exergonic)

Apply Your Knowledge: Use of K_{eq} to Determine ΔG°

- Hydrolysis of ATP: $ATP + H_2O \rightleftharpoons ADP + P_i + H^+$
- The relative concentrations of reactants have been determined for a reaction carried out at 25℃ and the value for K_{eq} was 2.23×10^{5}.
- Determine the standard free energy change?
- Is the reaction spontaneous or not? **<u>It is spontaneous</u>**

$$\Delta G^\circ = -RT \ln K'_{eq}$$

$$\Delta G^{\circ\prime} = (8.31\ \mathrm{J\ mol^{-1}\ K^{-1}})(298\ \mathrm{K})(12.32)$$

$$\Delta G^{\circ\prime} = -3.0500 \times 10^4\ \mathrm{J\ mol^{-1}} = -30.5\ \mathrm{kJ\ mol^{-1}} = -7.29\ \mathrm{kcal\ mol^{-1}}$$

Practice Problems

Use T= 25℃

glucose-6-phosphate isomerase

glucose-6-phosphate ⇌ fructose-6-phosphate

1. Given the K_{eq} = 0.075 in the above reaction, What is ΔG^{o} ?
   - Ans: +6.4KJ/mol or +6400J/mol

2. Find the K_{eq} of a reaction if ΔG^{o} = +2.0KJ/mol
   - Ans: 0.45

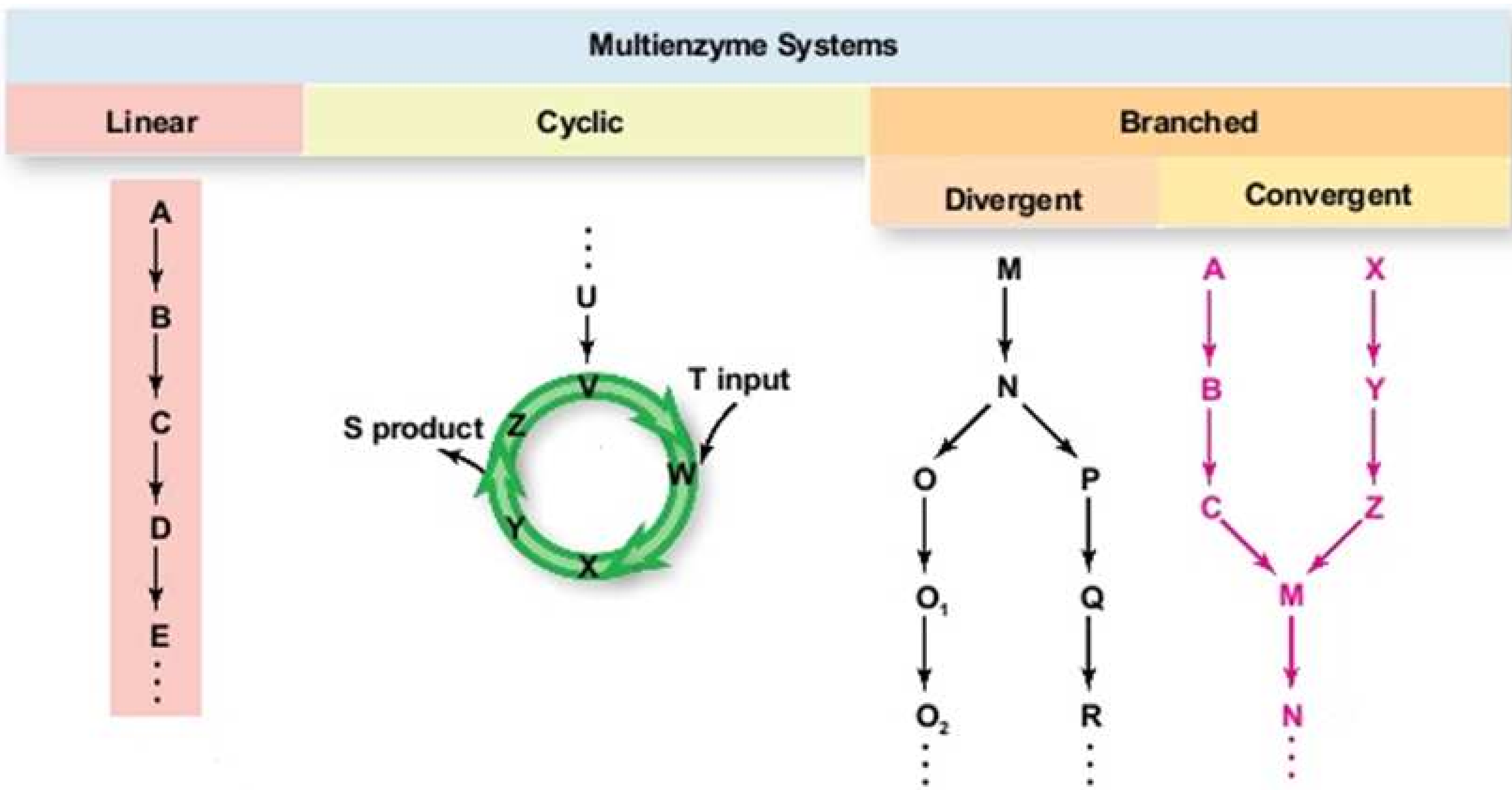
Metabolism Is the Sum of Cellular Reactions

- **Metabolism:** The entire network of chemical reactions carried out by living cells.
- **Anabolic reactions:** Synthesize molecules for cell maintenance, growth and reproduction.
- **Catabolic reactions:** Degrade molecules to create smaller molecules and energy.

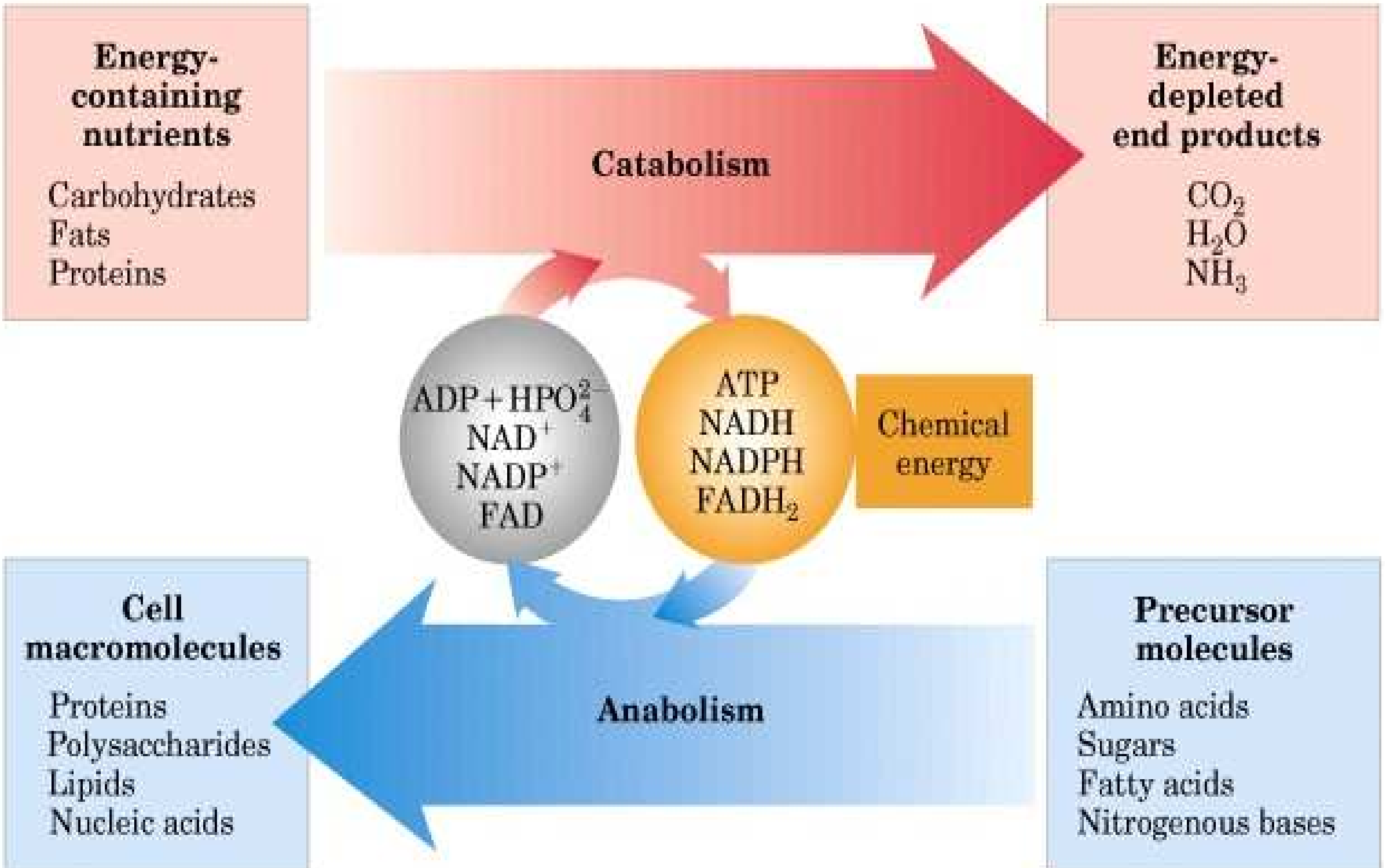
TERMINOLOGIES IN METABOLISM

- Catabolism
- Anabolism
- Amphibolic
- Intermediates
- Cataplerotic reactions
- Anaplerotic reactions
- Rate-limiting step
- Committed step

PATTERNS OF METABOLISM



COMPARISON OF CATABOLISM & ANABOLISM

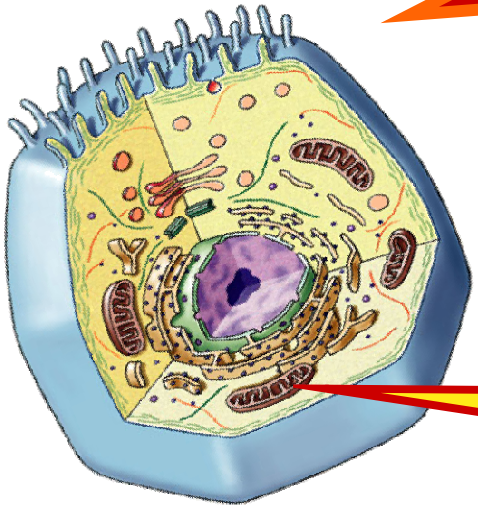


What do metabolic pathways accomplish?

- Generation of energy
- Synthesis of cellular building blocks
- Storage of energy
- Excretion/detoxification of harmful substances

Why is ATP referred as energy currency ?

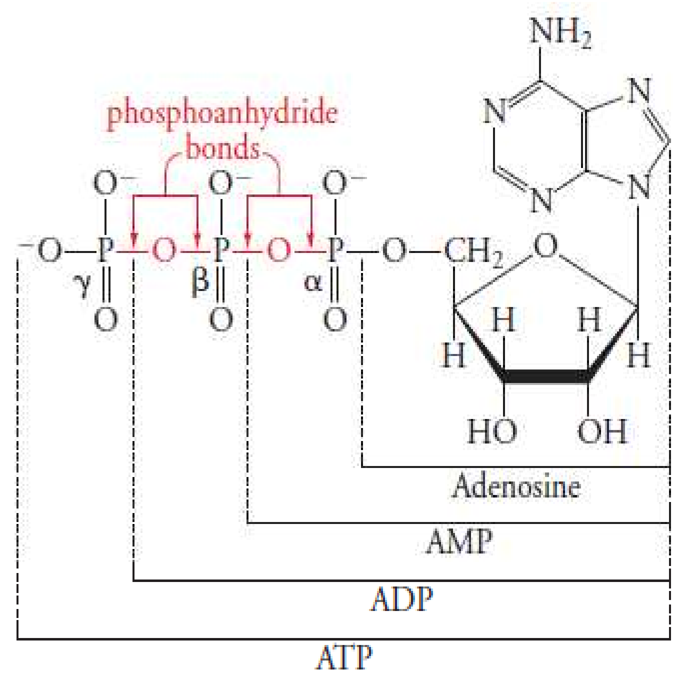
ATP

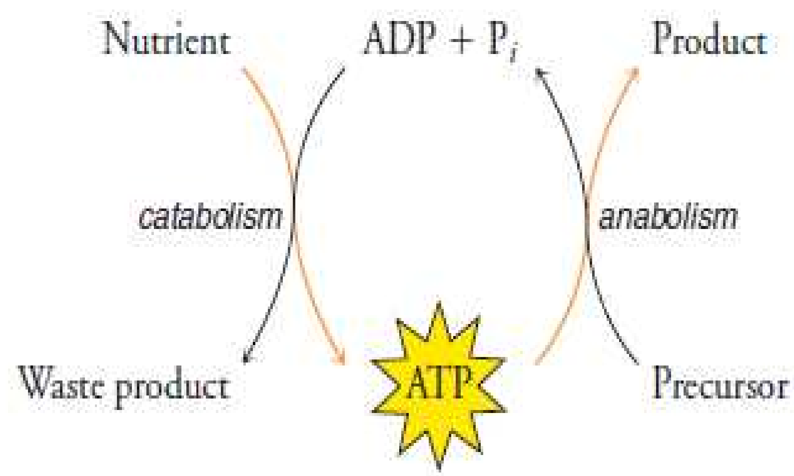


The point is to make
ATP

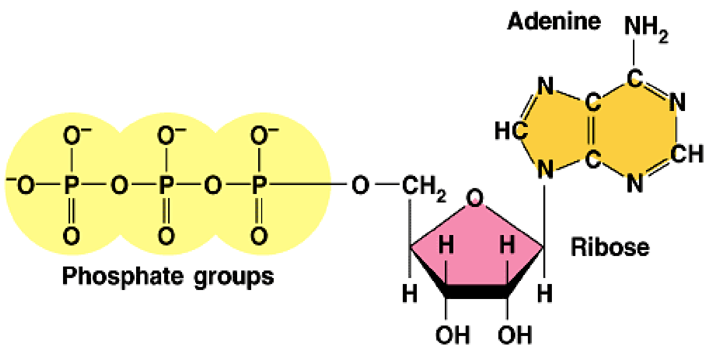
ATP: Energy Currency

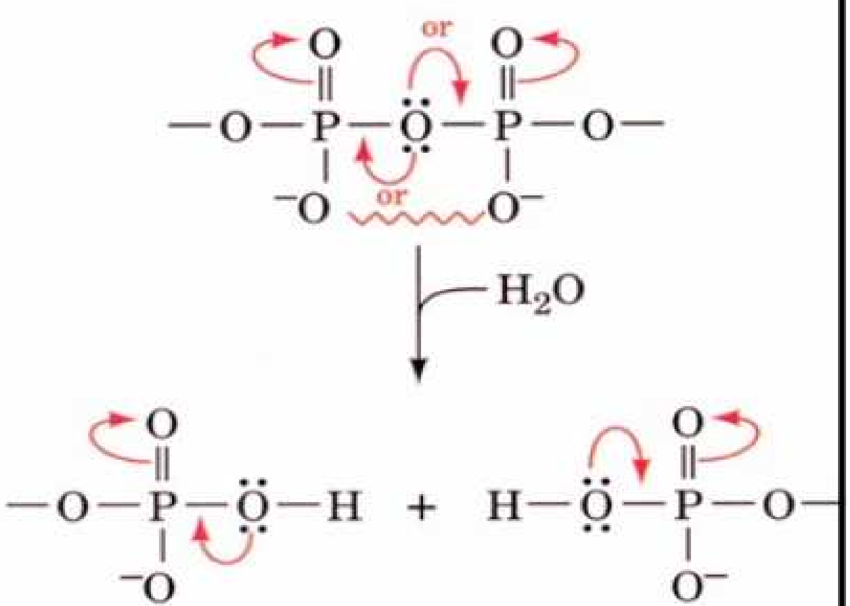
- Energy in nutrients doesn't transferred to cells rather releases and funnels through energy rich compound (ATP)
- ATP traps potential energy stored in macromolecules.
- ATP transfers energy to other compounds
- ATP appears to drive many thermodynamically unfavorable rxns





What makes ATP energetic?





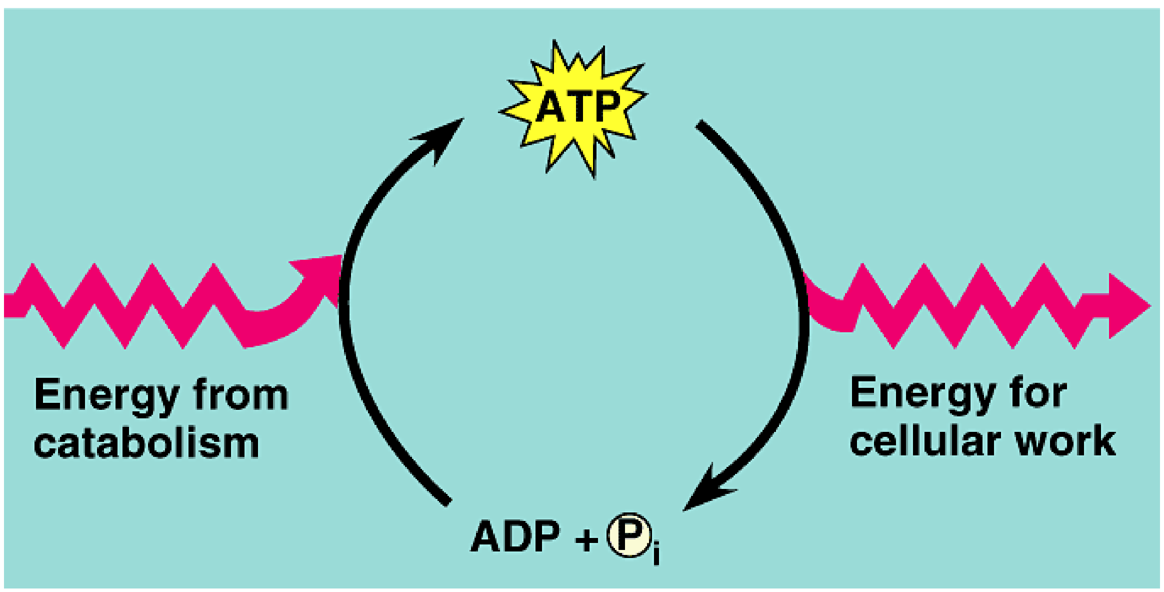
In phosphoanhydride, the P=O are each competing for the same anhydride oxygen lone pairs.

In the separated phosphates, there is no competition so the resonance is better.

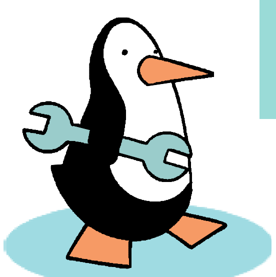
Finally, there is electrostatic repulsion between adjacent O- atoms in the phospho-anhydride (see zigzag line). This repulsion leads to destabilization of this form, favoring hydrolysis.

Is ATP Stored ?

- Cells can't store ATP
- ATP is good energy donor, not good energy storage
- Carbohydrates & fats are long term energy storage



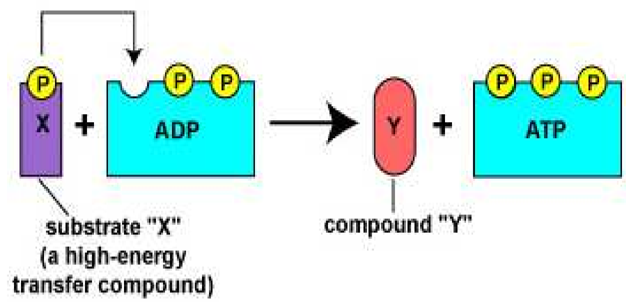
7.3 kcal/mole



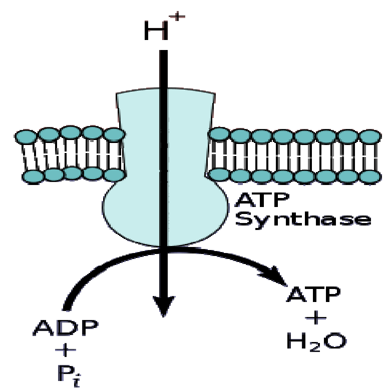
MECHANISMS OF ATP FORMATION

Two basic mechanisms

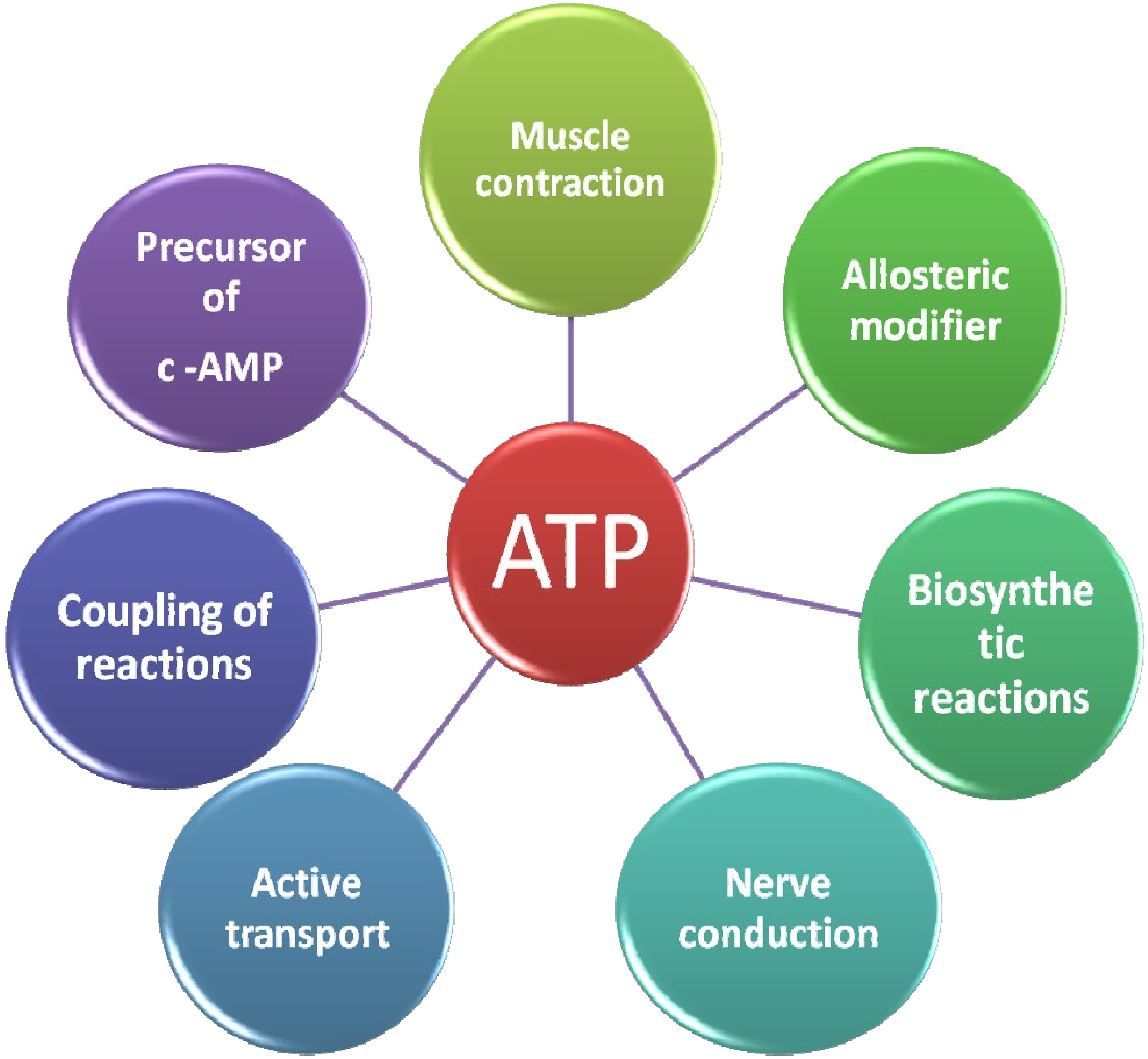
- Substrate level phosphorylation



- Oxidative phosphorylation



Functions of ATP



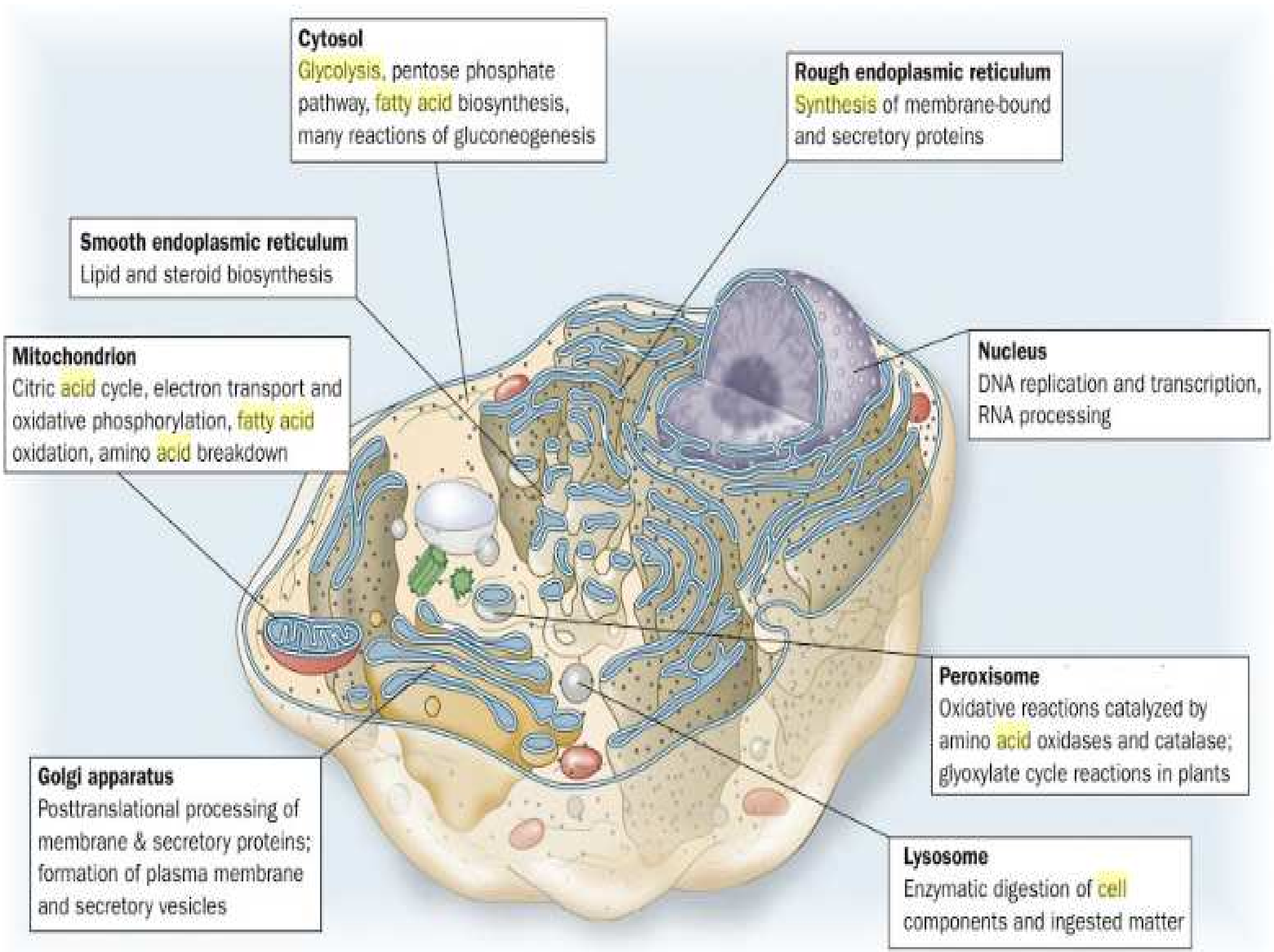
Major Bioenergetic Processes

- Glycolysis
- Gluconeogenesis
- Glycogenesis
- Glycogenolysis
- Pentose Phosphate Pathway
- Urea Cycle
- Fatty acid β-Oxidation
- TCA/Krebs Cycle
- Electro transport Chain
- Oxidative Phosphorylation

COMMON PRINCIPLES TO METABOLIC PATHWAYS

- Many metabolic reactions involve oxidation or reduction
- Metabolic pathways contain reversible & irreversible steps
- Metabolic pathways are interconnected
- Metabolic pathways are not necessarily linear.
- Metabolic pathways are localized to compartments in cell
- Different repertoire of pathways occurs in different organs
- Different metabolic processes occur in fed & fasting state
- Metabolic pathways are regulated

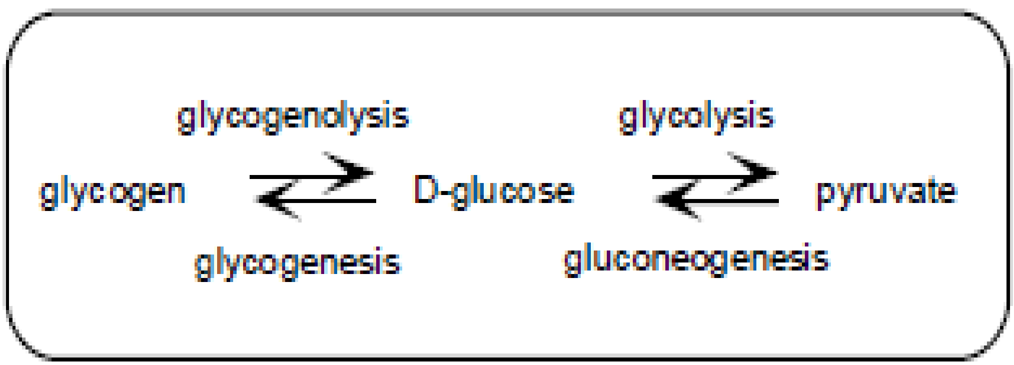
Metabolic Functions of Eukaryotic Organelles



Regulation of Metabolic Pathways

- Substrate availability
- Product Inhibition
- Allosteric regulation
- Covalent modification
- Alteration in transcription of gene

CARBOHYDRATE METABOLISM



Carbohydrates Digestion and absorption

- Carbohydrate metabolism focuses on the synthesis and usage of glucose, a major fuel for organisms.
- Carbohydrate can be synthesized from lactate, glycerol and amino acids but most carbohydrate is derived ultimately from plants.
- Ingested carbohydrates are converted to smaller chains by salivary and pancreatic amylase. In addition intestinal hydrolytic enzymes convert them to monosaccharide's then enter intestinal cells and are transported to the liver or other tissues.

Sucrose $\xrightarrow{\text{Sucrase}}$ Glucose + Fructose

* Ruminants do not have sucrase

Maltose $\xrightarrow{\text{Maltase}}$ Glucose + Glucose

Lactose $\xrightarrow{\text{Lactase}}$ Glucose + Galactose

* Poultry do not have lactase

- Cellulose is digested by symbiotic bacteria and ciliates living in the gut of herbivorous animals (ruminants) by action of the enzyme cellulase.
- Ruminants digest carbohydrates to volatile fatty acids (VFA's) which later metabolized by the host to produce energy yielding compounds.
    - Acetate
    - Propionate
    - Butyrate

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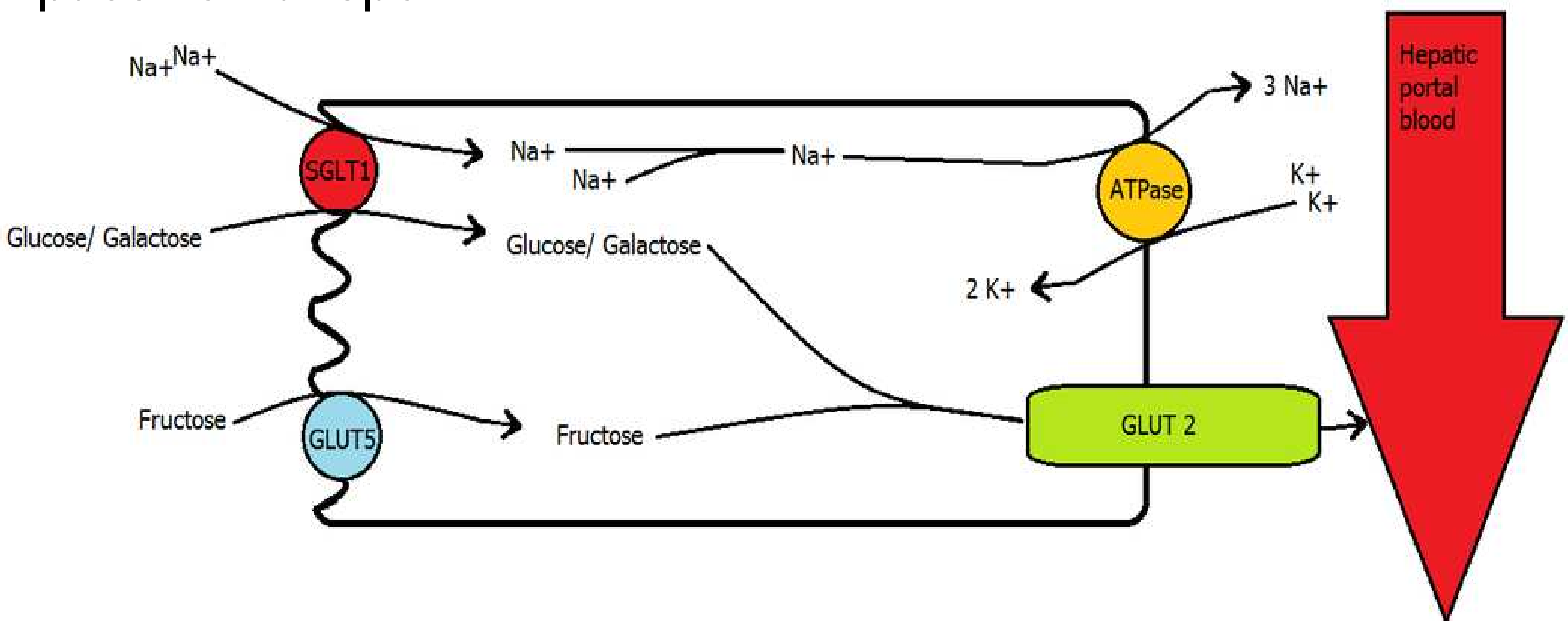
- Monosaccharides glucose & galactose are transported into the epithelial cells by common protein carriers via secondary active transport.
- Fructose chemically different from glucose and galactose enters into the cells by facilitated diffusion, also called passive transport.

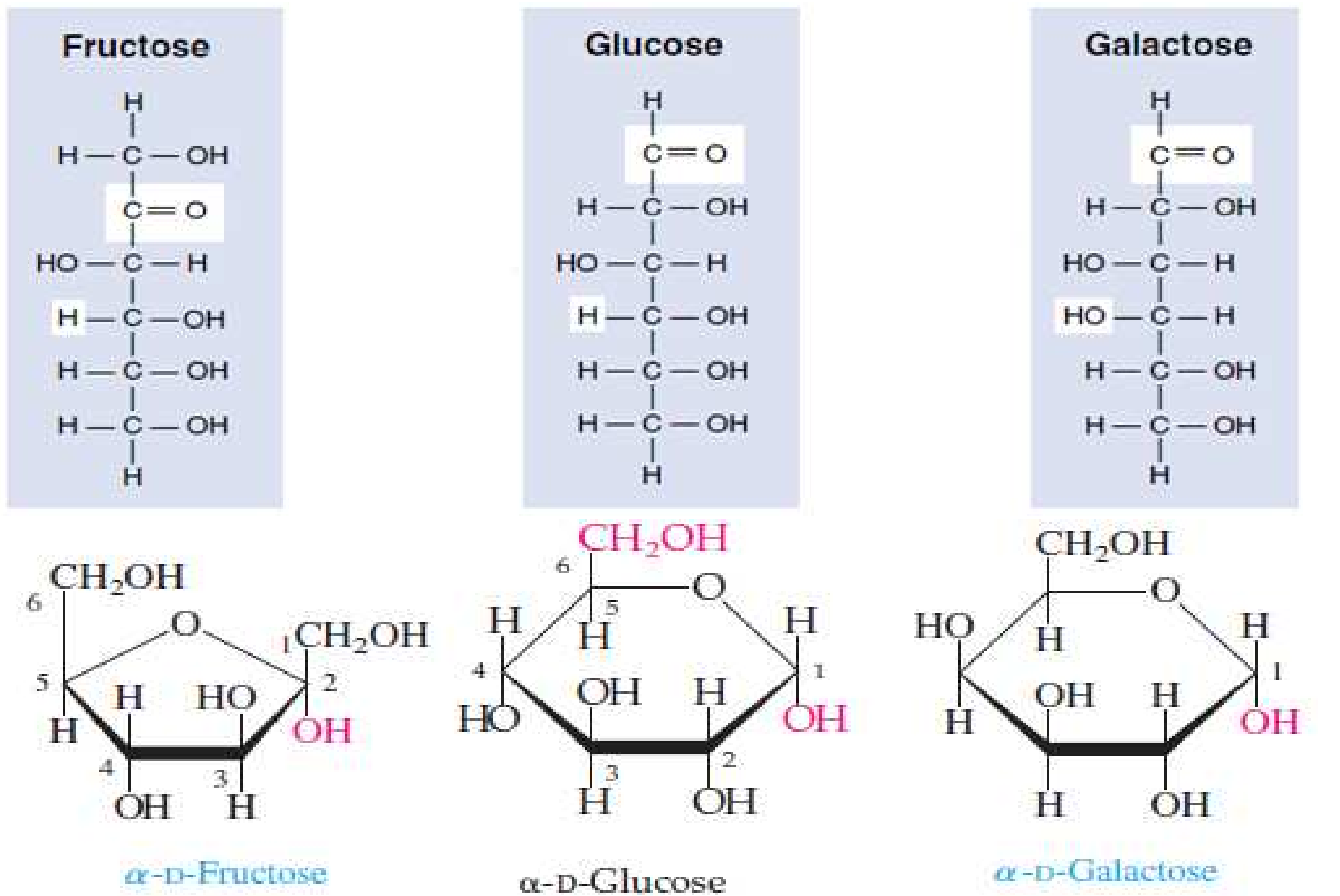


WHY IS FRUCTOSE USES DIFFERENT PATH

UNLIKE GLUCOSE & GALACTOSE ?

Wilson & Crane Hypothesis

For active transport to take place the structure of monosaccharides should be hexose ring and the OH group at position 2 should be right side. Fructose doesn't have it.



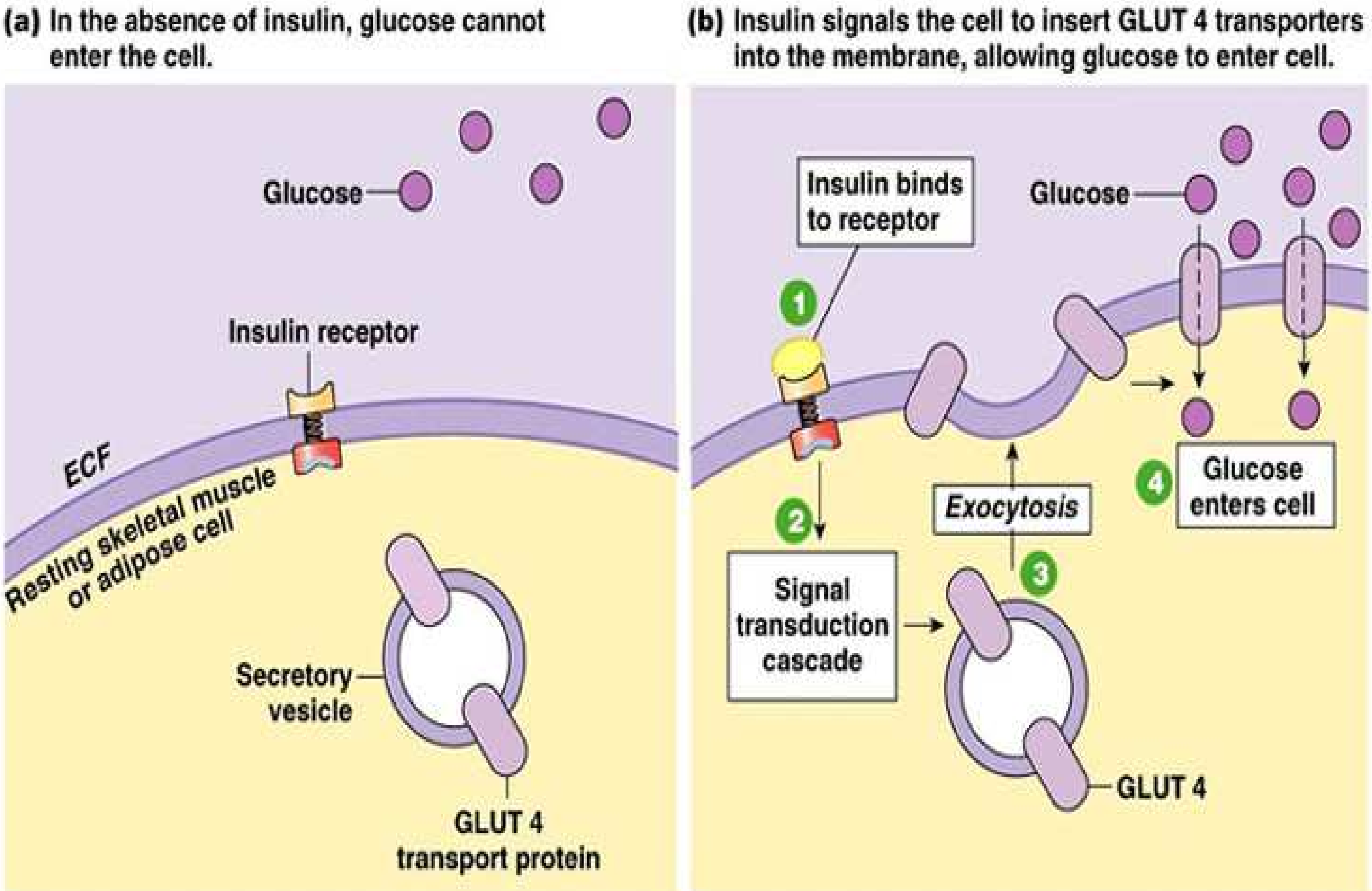
Glycolysis

- Glycolysis, also referred to as the Embden-Meyerhof-Parnas pathway
- Glycolysis is a metabolic pathway that occurs in cytosol and cleaves glucose into two molecules of pyruvate or lactate.
- The main function of glycolysis is energy (ATP) production.
- It can occur in aerobic or anaerobic environment.

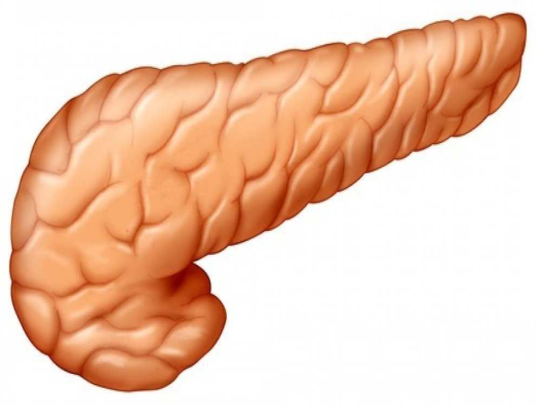
UPTAKE OF GLUCOSE INTO CELLS

- Glucose transporters (GLUT) facilitates movement of glucose across the plasma membrane which are designated as GLUT1-14 (Isoforms).
- GLUT1, GLUT2 & GLUT3 are insulin independent.
- Following insulin stimulation,GLUT4-containing vesicles in muscle and adipocytes translocation to and fuse with the plasma membrane, thus providing the mechanism by which insulin stimulates uptake of glucose from the blood.

Insulin Mediated Glucose Transport



What makes the insulin to be released from the pancreas ??



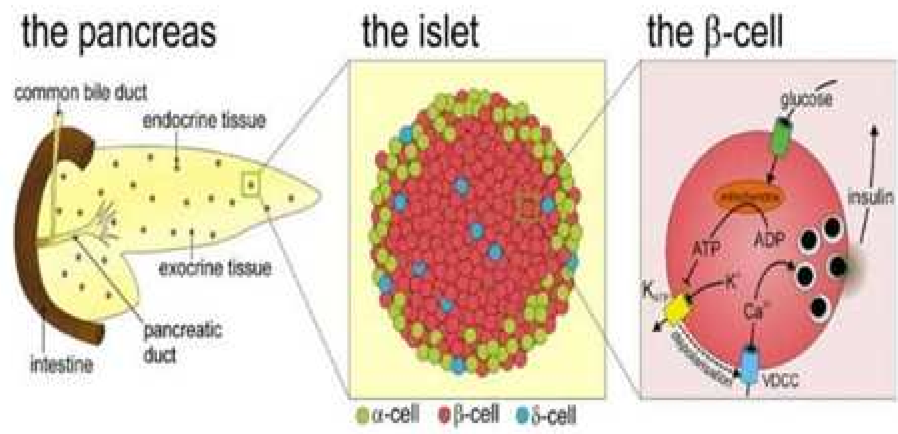
1. Gastrointestinal hormones

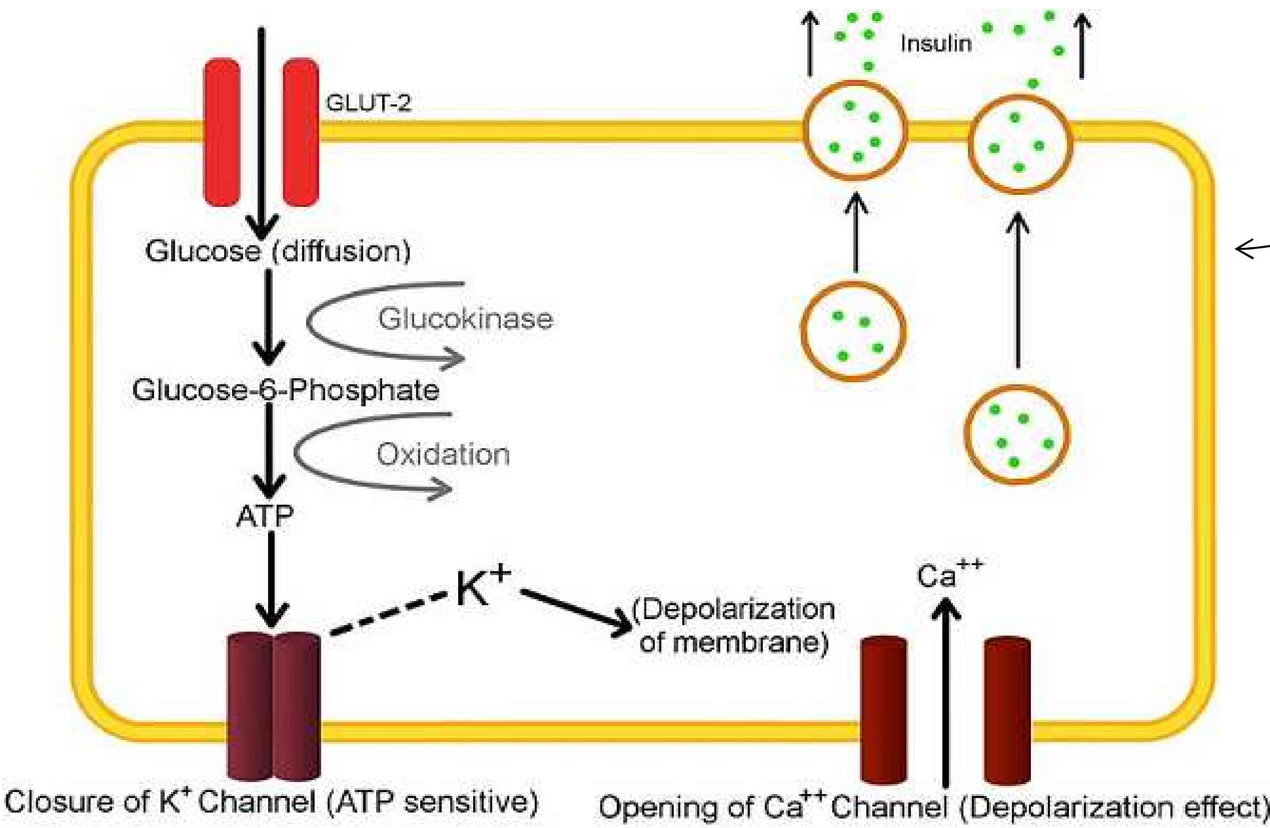
- The intestinal peptides
  - Cholecystokinin (CCK)
  - Glucose-dependent insulinotropic peptide (GIP)
  - Glucagon like peptide (GLP)
- They are released from the entroendocrine cells after the ingestion and cause an anticipatory rise in insulin levels.

2. Glucose in blood stream

- The glucose that goes in blood stream passively diffuses to β-cell of pancreas through GLUT-2; glucose gets converted subsequently oxidized to form ATP.
- This process inhibits the ATP-sensitive K^+ channels to close. The closure of the ATP sensitive K^+ channel causes depolarization of the cell membrane causing the cell to open Ca^{+2} channel.
- The influx of Ca^{+2} then stimulates fusion of the insulin vesicles to the cell membrane and secretion of insulin.

INSULIN SECRETION BY B-CELLS OF PANCREAS

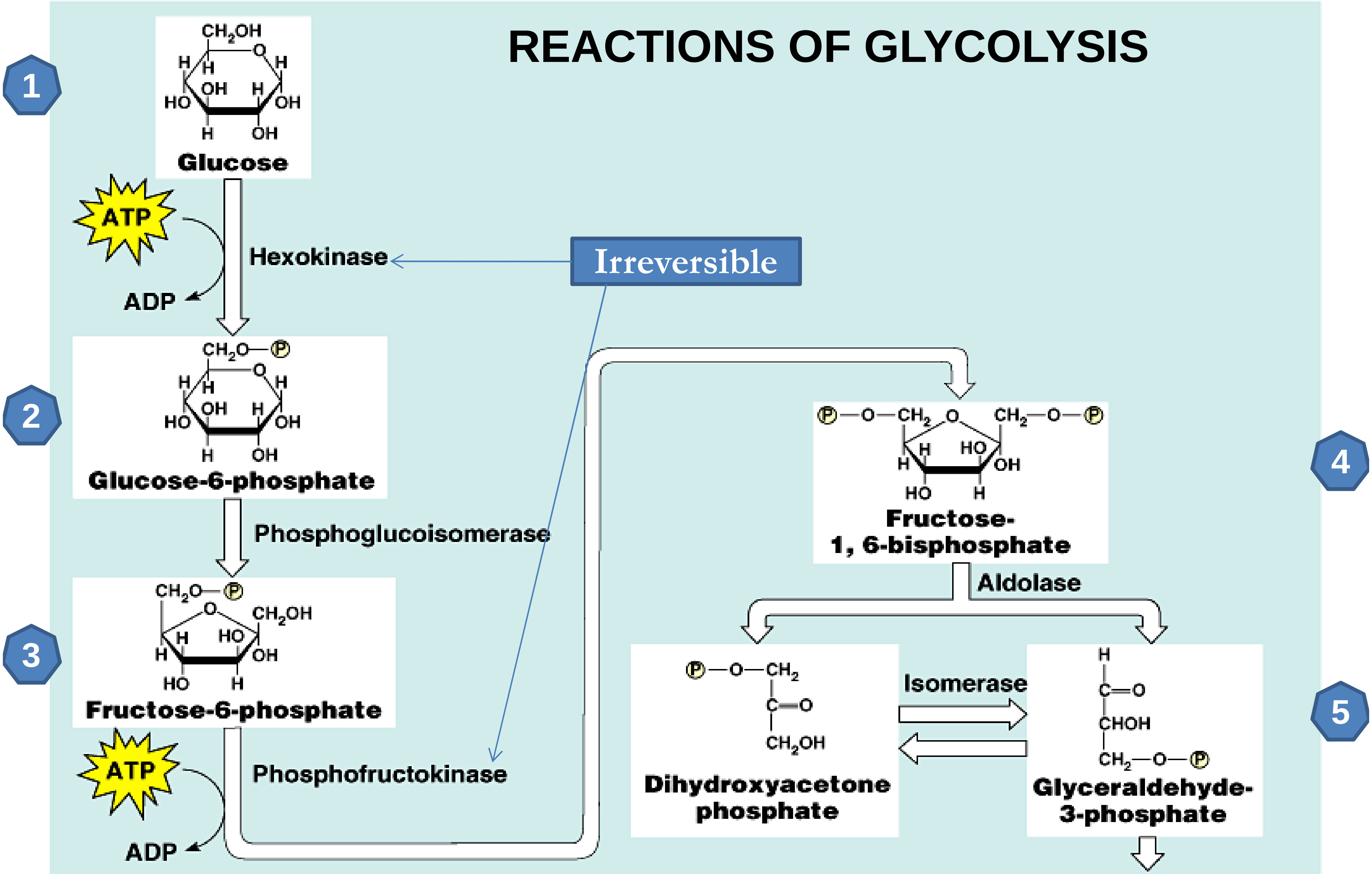




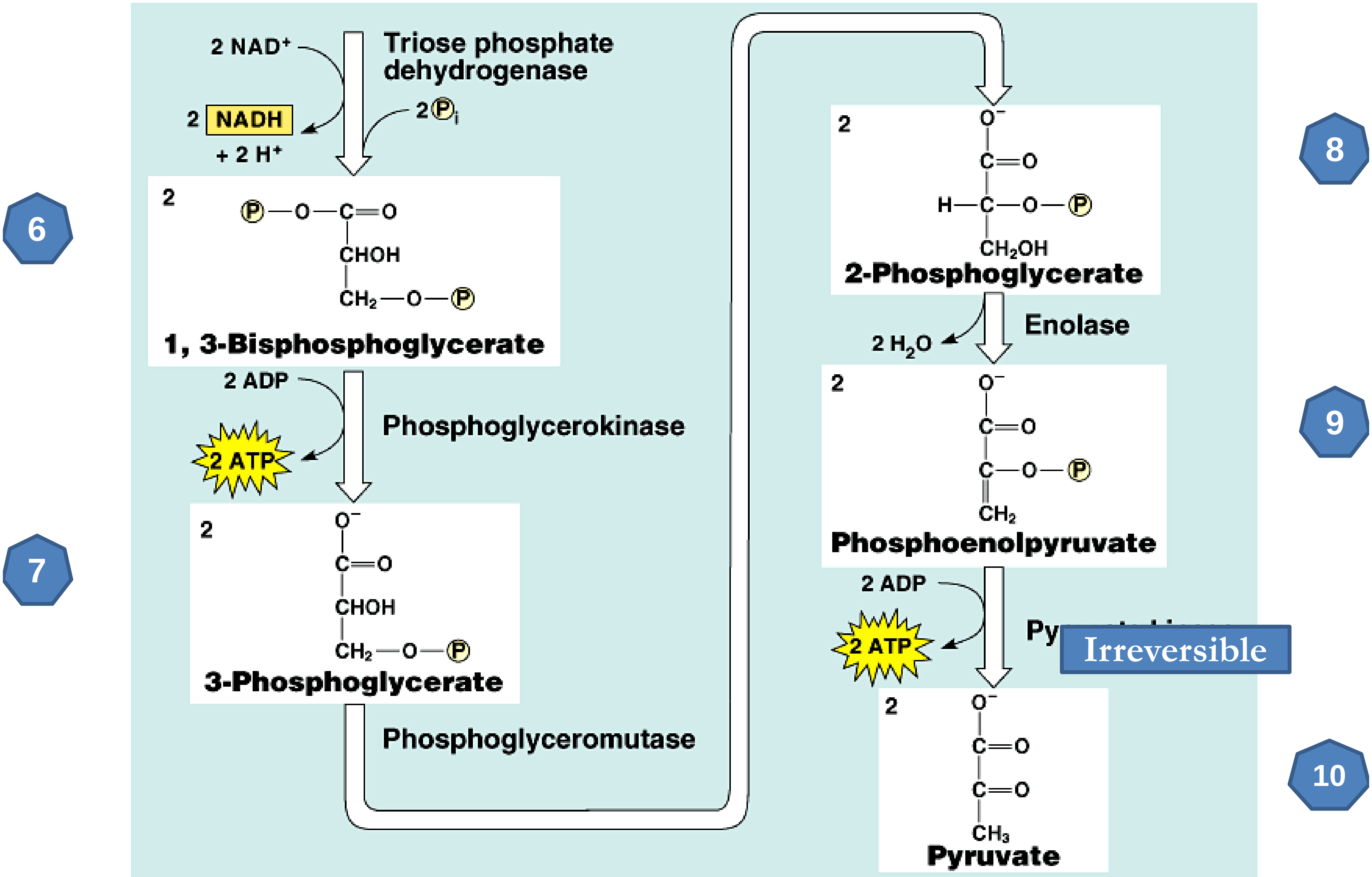
PHASES OF GLYCOLYSIS

The entire glycolysis pathway can be separated into two phases:

- **Preparatory phase**
  - ATP is consumed
- **Pay-off phase**
  - ATP is produced.



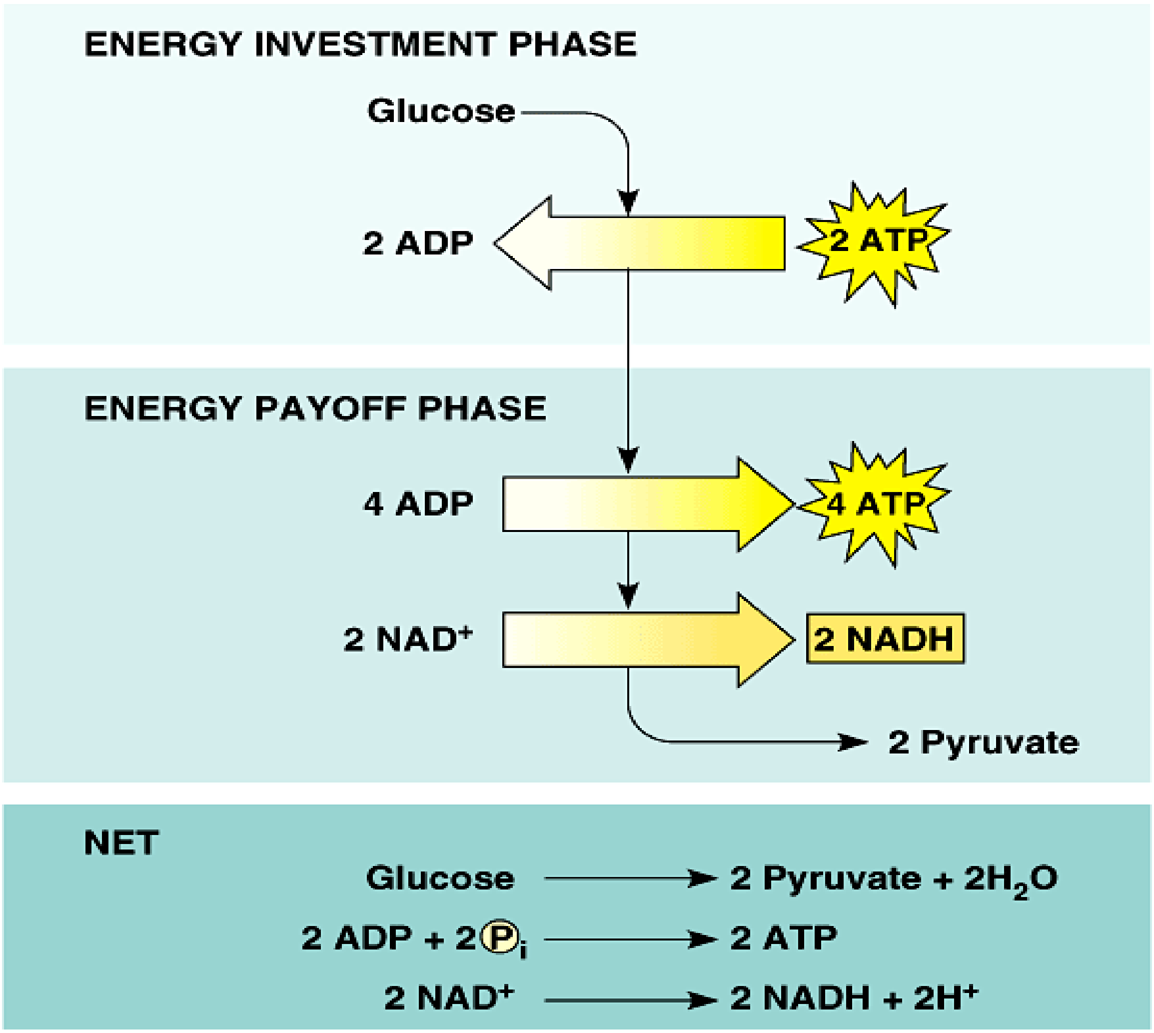
Energy Investment Phase (Steps 1-5)



Energy-Payoff Phase (Steps 6-10)

Step	Enzyme	Reaction
1	Hexokinase	Uses ATP to phosphorylate glucose, increasing its potential energy.
2	Phosphoglucose isomerase	Converts glucose-6-phosphate to fructose-6-phosphate; referred to as an isomer of glucose-6-phosphate.
3	Phosphofructokinase	Uses ATP to phosphorylate the opposite end of fructose-6-phosphate, increasing its potential energy.
4	Fructose-bis-phosphate aldolase	Cleaves fructose-1,6-bisphosphate into two different three-carbon sugars.
5	Triose phosphate isomerase	Converts dihydroxyacetone phosphate (DAP) to glyceraldehyde-3-phosphate (G3P). Although the reaction is fully reversible, the DAP-to-G3P reaction is favored because G3P is immediately used as a substrate for step 6.
6	Glyceraldehyde-3-phosphate dehydrogenase	A two-step reaction that first oxidizes G3P using the **NAD^+** coenzyme to produce NADH. Energy from this reaction is used to attach a P_i to the oxidized product to form 1,3-bisphosphoglycerate.
7	Phosphoglycerate kinase	Transfers a phosphate from 1,3-bisphosphoglycerate to **ADP** to make 3-phosphoglycerate and ATP.
8	Phosphoglycerate mutase	Rearranges the phosphate in 3-phosphoglycerate to make 2-phosphoglycerate.
9	Enolase	Removes a water molecule from 2-phosphoglycerate to form a C=C double bond and produce phosphoenolpyruvate.
10	Pyruvate kinase	Transfers a phosphate from phosphoenolpyruvate to **ADP** to make pyruvate and ATP.

Summary of the Glycolysis

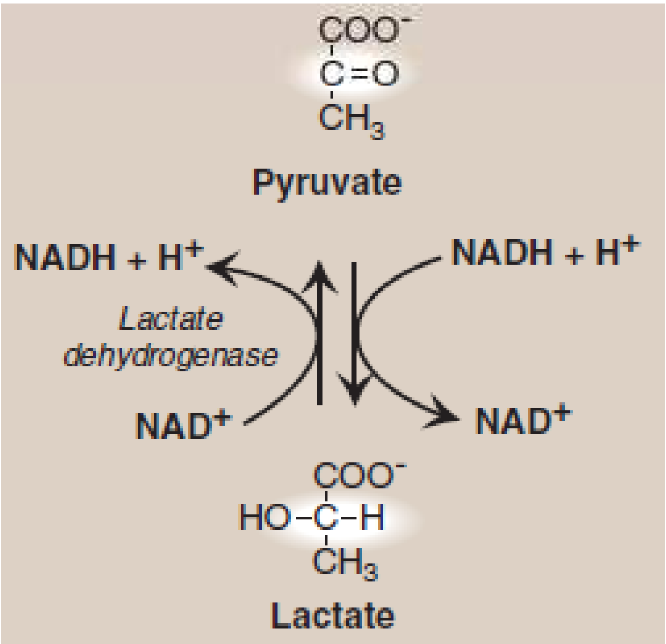


Comparison of Hexokinase & Glucokinase

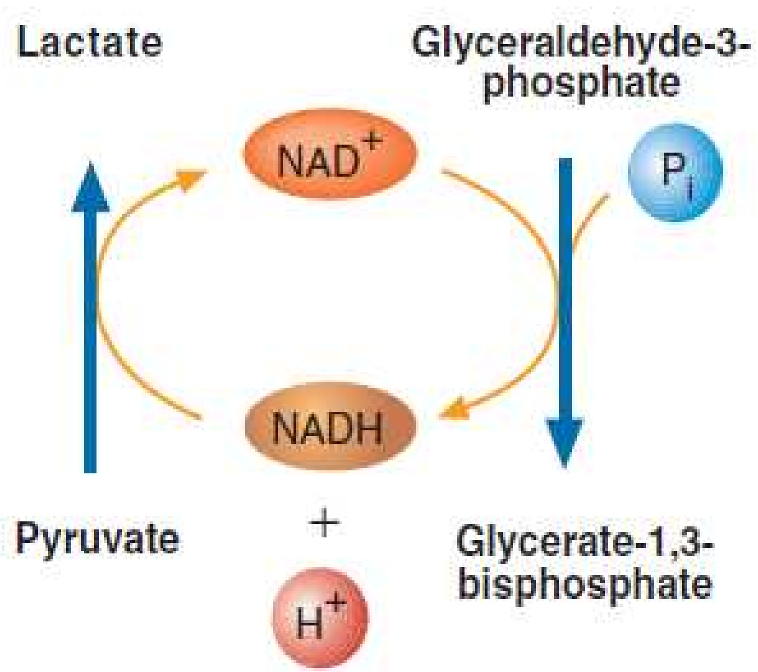
Hexokinase	Glucokinase
• High affinity for substrate	• Low affinity for substrate
• Expressed in all tissues	• Expressed primarily in liver & pancreatic cells
• Phosphorylates variety of hexose sugars	• Highly specific for glucose
• Inhibited by G-6-P	• Not inhibited to G-6-P.
• Not induced by insulin	• Induced by insulin

Fates of NADH and Pyruvate

- In aerobic glycolysis
  - NADH reoxidized via the ETC.
  - Pyruvate enters the TCA cycle.
- In anaerobic glycolysis
  - NADH reoxidized by conversion of pyruvate to lactate by lactate dehydrogenase.



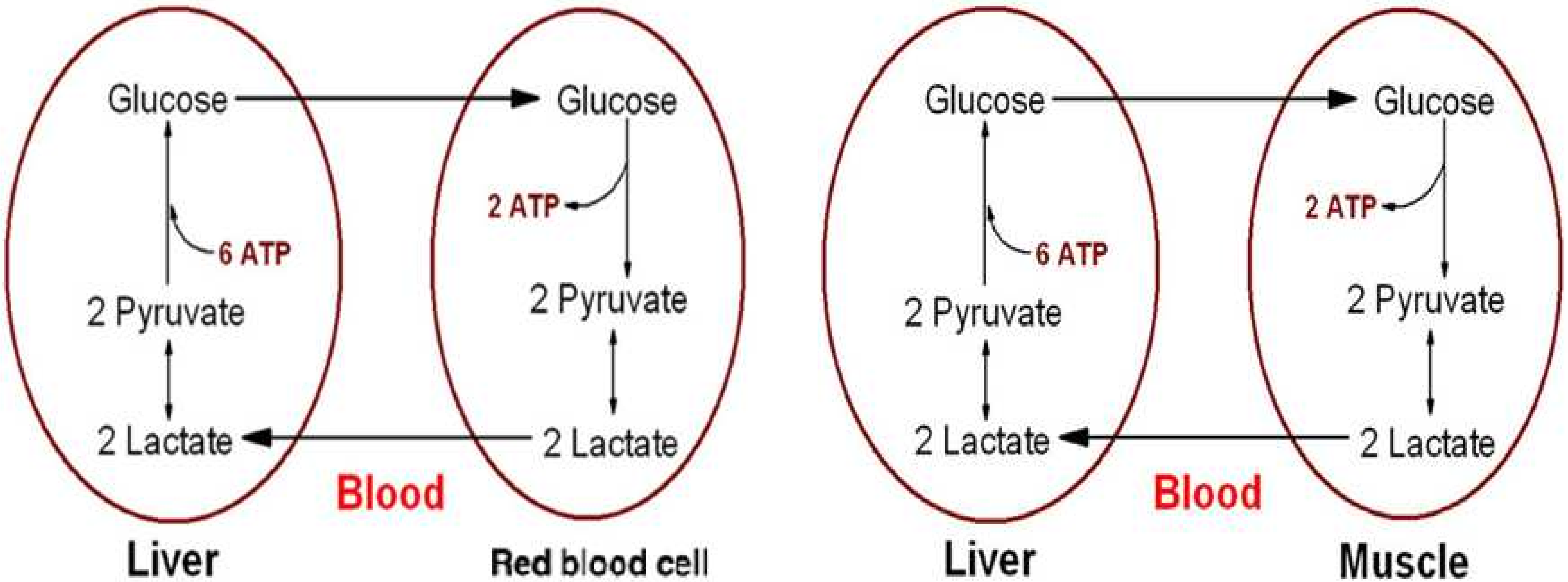
- Thus reduction of pyruvate to lactate is coupled to the oxidation of G-3-P to glycerate-1,3-bisphosphate
  - **Refer 6th step of glycolysis.**



Lactate Metabolism & Cori Cycle

- The cycle in carbohydrate metabolism consisting of the conversion of glucose to lactate in RBCs and muscle, diffusion of the lactate into the bloodstream which carries it to the liver where it is converted into glucose, which is transported to RBCs & muscle (reconverted into lactate).
- The pathway operates when there is inadequate oxygen supply, typically the result of any intense muscular activity such as running or in cells lacking mitochondria; energy is released through "anaerobic metabolism".

Cori Cycle



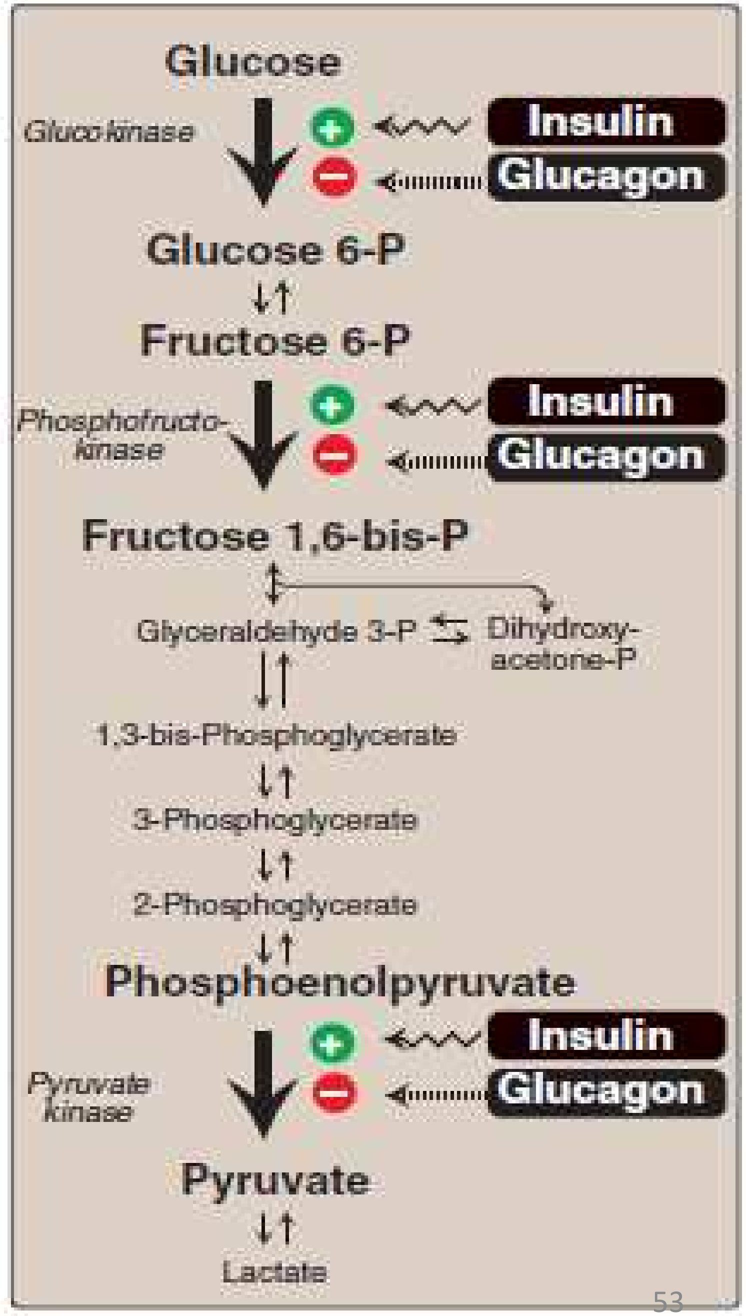
Glucose
Glucose
2 ATP
6 ATP
2 Pyruvate
2 Pyruvate
2 Lactate
2 Lactate
Blood
Liver
Red blood cell
Glucose
Glucose
2 ATP
6 ATP
2 Pyruvate
2 Pyruvate
2 Lactate
2 Lactate
Blood
Liver
Muscle

Regulation of Glycolysis

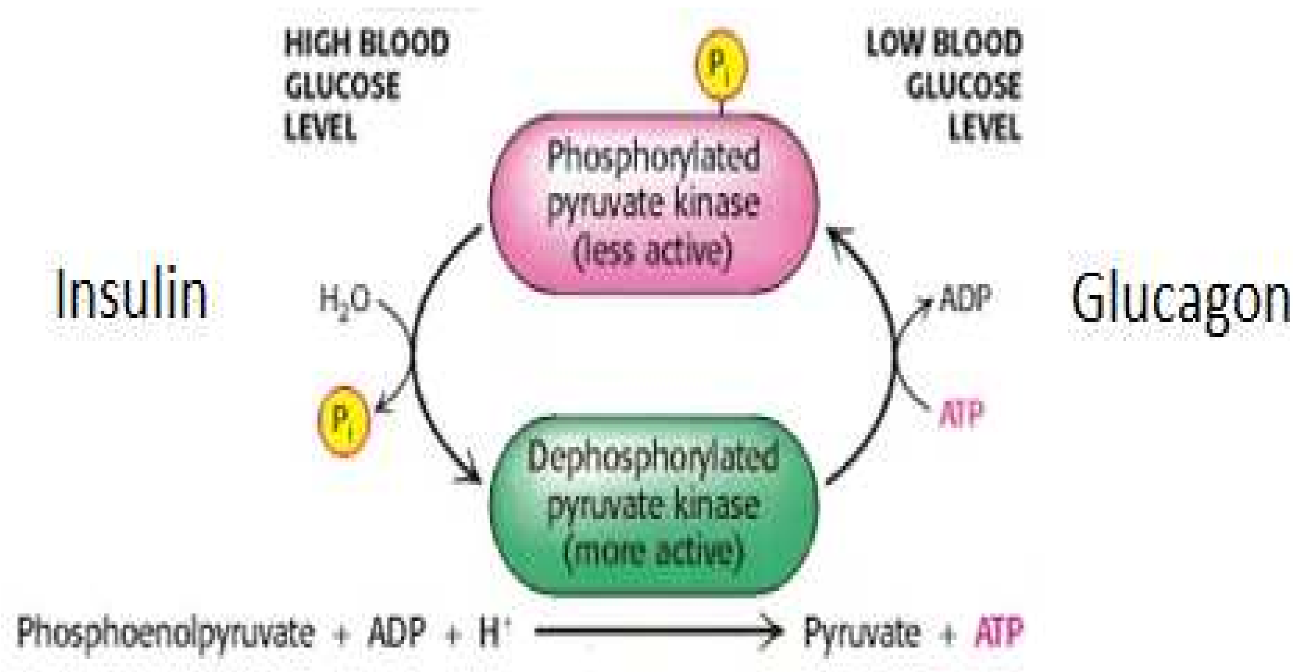
- The rate of conversion of glucose in to pyruvate is regulated to meet the cellular needs. In many metabolic pathways enzymes catalyzing essentially irreversible reactions are potential sites for control.
- In glycolysis the reactions catalyzed by
  - Hexokinase
    - Inhibited by G-6-P
  - Phosphofructokinase
    - Inhibited by ATP & activated by AMP
  - Pyruvate kinase
    - Inhibited by ATP & Acetyl-CoA

Hormonal Regulation

- **Insulin** initiates an increase in the amount of glucokinase, PFK, & pyruvate kinase in liver.
  - These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis.
- Conversely, gene transcription & synthesis of glucokinase, PFK, & pyruvate kinase are decreased by **glucagon.**



- **Covalent Modification**
  - Glucagon inactivates pyruvate kinase through phosphorylation (less active).
  - Insulin activates through dephosphorylation (more active); this increase conversion of PEP to pyruvate, which has the effect to the synthesis of ATP.



Entry of other Monosaccharide's in to glycolytic pathway

- Monosaccharides besides glucose meet their catabolic fate in glycolysis, after being transformed into one of the glycolytic intermediates.
- This is because the broad specificity of Hexokinases.
  - Galactose
  - Mannose
  - Fructose

Galactose

- Galactose converted to galactose-1-phosphate by galactokinase then transformation to glucose-1-phosphate by galactose-4- epimerase.
- Glucose-1-phosphate is changed to glucose-6-phosphate by phosphoglucomutase.

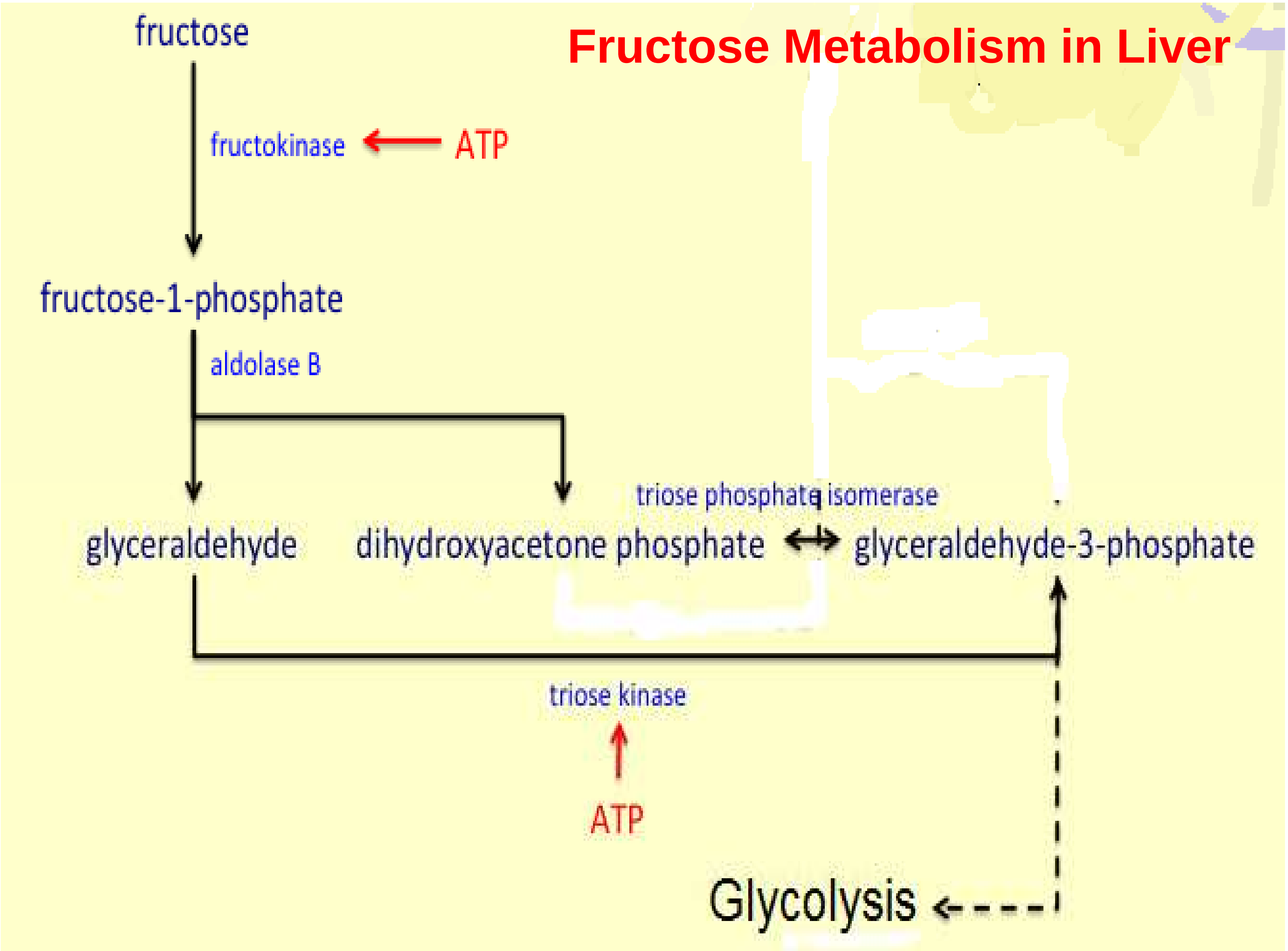
Mannose

- Mannose utilization is by hexokinase to mannose-6-phosphate followed by isomerization of mannose-6-phosphate to fructose-6-phosphate by manose-6-phosphate isomerase.

Fructose

- In most tissues fructose is phosphorylated by hexokinase and becomes Fructose-6-phosphate (F-6-P)
  - Major pathway of fructose entry into glycolysis
- In liver fructose enters by a different pathway. The liver enzyme fructokinase catalyzes the phosphorylation of fructose and becomes Fructose-1-phosphate (F-1-P).

- F-1-P cleaved to glyceraldehyde & dihydroxyacetone phosphate by F-1-P aldolase.
  - Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate (G-3-P) by the glycolytic enzyme triose phosphate isomerase.
  - Glyceraldehyde is phosphorylated to G-3-P by triose kinase in presence of ATP.
- Thus both products of F-1-P hydrolysis enter the glycolytic pathway as G-3-P.


Fructose Metabolism in Liver
fructose
fructokinase
ATP
fructose-1-phosphate
aldolase B
glyceraldehyde
dihydroxyacetone phosphate
triose phosphate isomerase
glyceraldehyde-3-phosphate
triose kinase
ATP
Glycolysis

SUMMARY OF GLYCOLYSIS

Entering substrates	Glucose and other monosaccharides
Enzyme location	Cytosol
Net ATP production	2 ATP formed directly per molecule of glucose entering pathway Can be produced in the absence of oxygen (anaerobically)
Coenzyme production	2 NADH + 2 H^+ formed under aerobic conditions
Final products	Pyruvate—under aerobic conditions Lactate—under anaerobic conditions
Net reaction	
Aerobic:	Glucose + 2 ADP + 2 P_i + 2 NAD^+ $\longrightarrow$ 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O
Anaerobic:	Glucose + 2 ADP + 2 P_i $\longrightarrow$ 2 lactate + 2 ATP + 2 H_2O