BCHM 270: Module 4

CARBOHYDRATE METABOLISM

Agenda

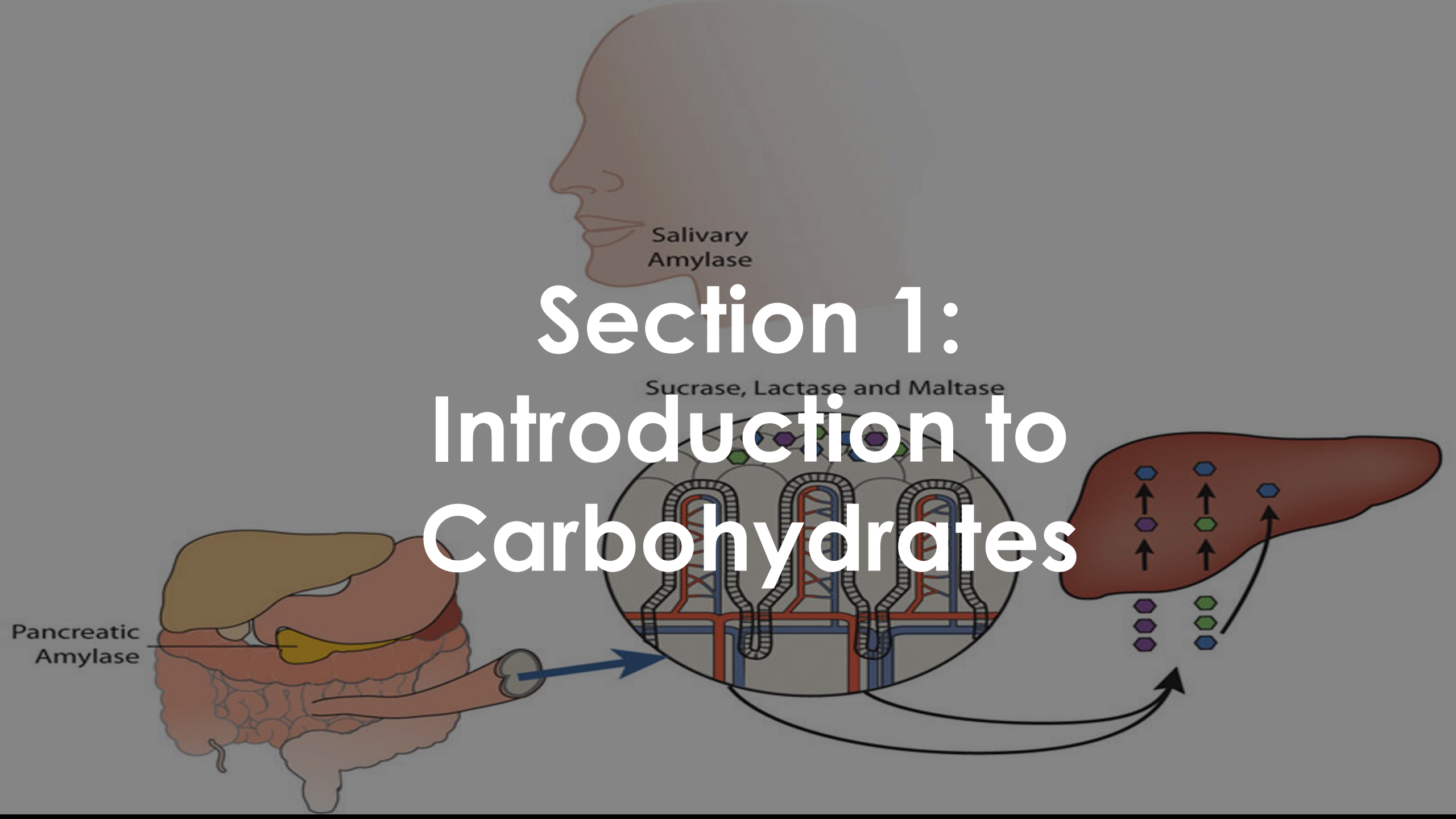
Section 1..... Introduction to Carbohydrates

Section 2..... Glycolysis

Section 3..... Preparing for the TCA Cycle

Section 4..... Gluconeogenesis and Glycogen Metabolism

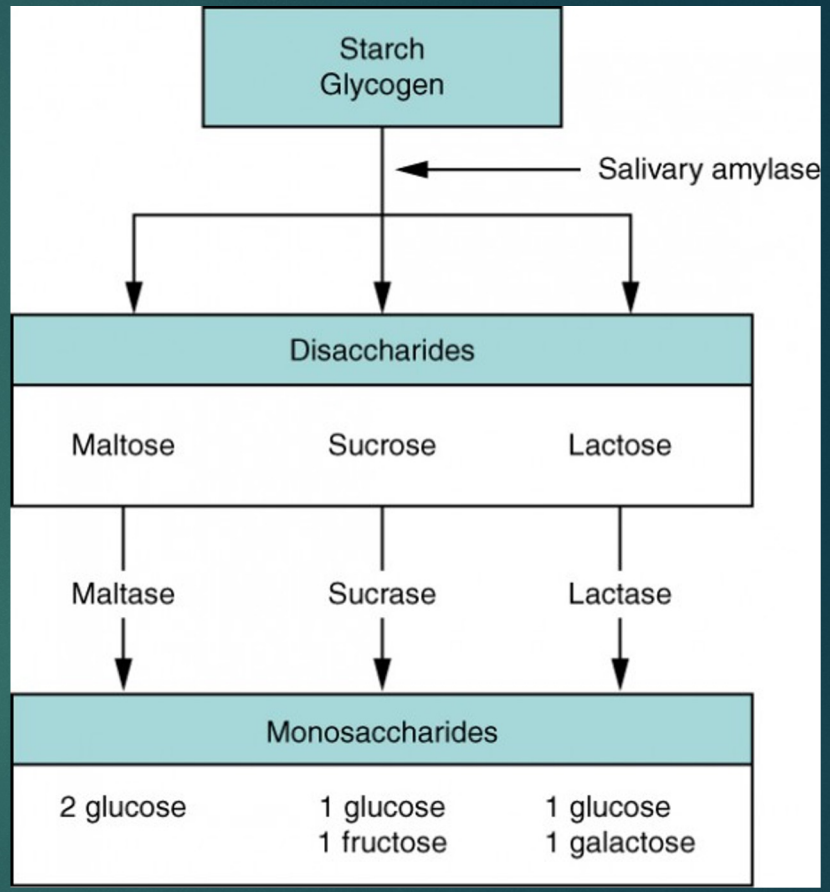
Section 5..... Pentose Phosphate Pathway



Section 1: Introduction to Carbohydrates

Concept 1.1: Digestion of Carbohydrates

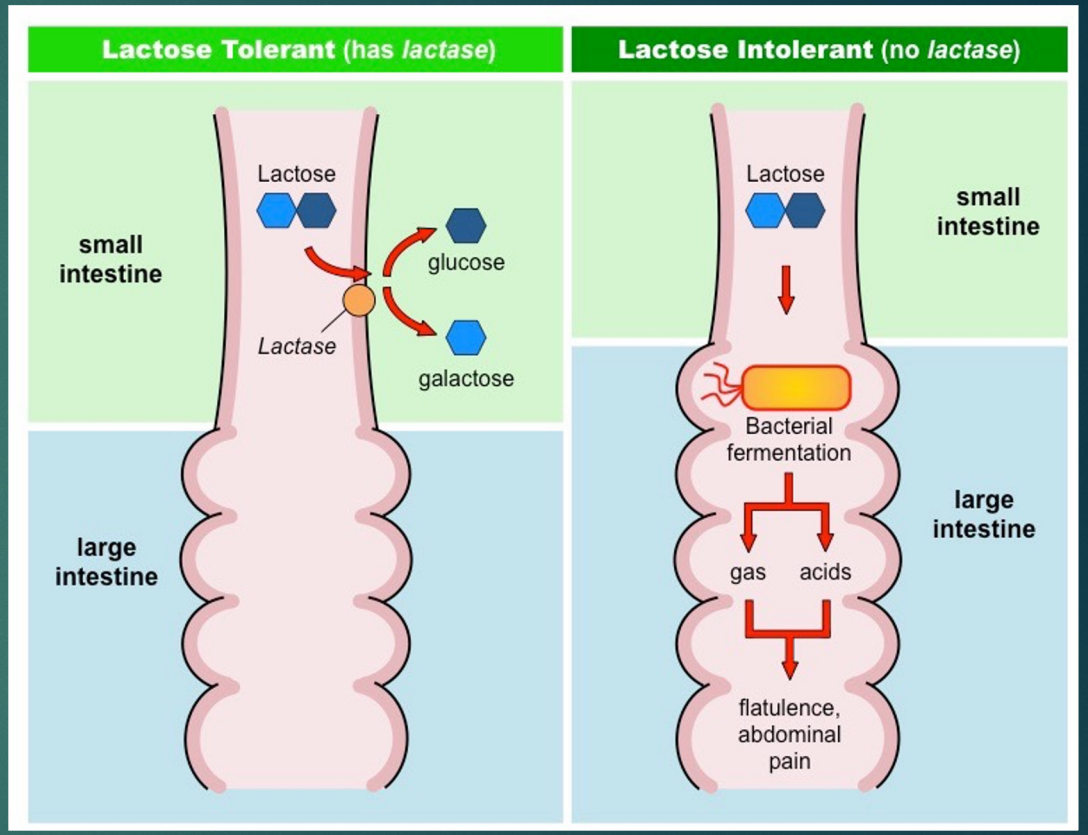
- **Glycosidases:** enzymes that cleave glycosidic bonds with water (hydrolysis)
- Sources of digestion
  - Mouth
    - α-amylase released by salivary glands -> cleaves α-1,4 bonds, producing branched oligosaccharides
    - Humans only produce α-1,4 endoglycosidases -> cannot digest cellulose because of β-1,4 bonds
    - Carbohydrate digestion stops in stomach (pH too low)
  - Intestines
    - Pancreatic α-amylase continues digestion
    - Brush border enzymes specific to disaccharides (e.g. maltase, sucrase, lactase) from intestinal mucosal cells in jejunum finish digestion
    - Absorption into blood occurs in duodenum, upper jejunum



Breakdown of carbohydrates.

Spotlight on Disease, Lactose Intolerance

- Digestive enzyme deficiency etiology:
  - Genetic deficiencies in enzymes catalyzing final disaccharide cleavage in the intestine, malnutrition, Intestinal diseases, drugs damaging intestinal mucosa
- Lactose intolerance:
  - Lactose metabolized by bacteria in the large intestine
    - Loss of bush border enzymes can result in a lack of enzyme leading to bloating, diarrhea, and dehydration when dairy sources are consumed (high lactose)
      - Treat by removing lactose from diet or reintroducing lactase



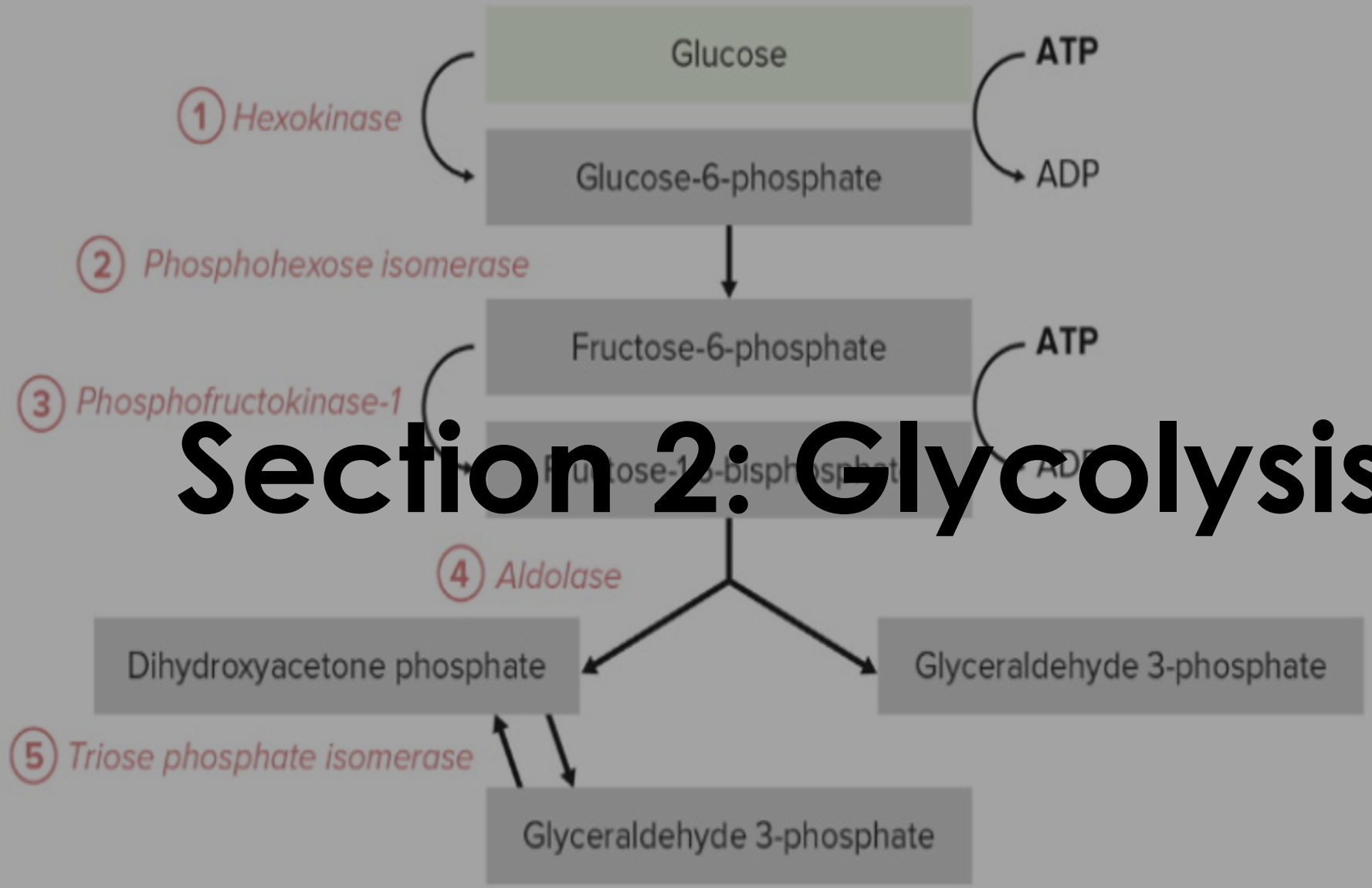
Pathophysiology of Lactose Intolerance.

Section 1 Quiz

Q1. Humans cannot digest cellulose because they lack?

a. β-amylase
b. α-amylase
c. Lactase
d. Maltase

Answer: A



Section 2: Glycolysis

Concept 2.1: Introduction to Glycolysis

- Catabolic process that converts 1 glucose into 2 pyruvate
- Only energy production pathway in cells in some cells (red blood cells, sperm)
  - Energy investment phase (First 5 reactions):
    - 2 ATP used
  - Energy generation phase (Last 5 reactions):
    - 2 NADH produced ◦ 4 ATP produced
  - Net glycolysis products:
    - ◦ 2 pyruvate ◦ 2 NADH ◦ 2 ATP

$$glucose(6C) + 2NAD + 2ADP + 2inorganicphosphates(P_i)$$

$$\rightarrow 2pyruvate(3C) + 2NADH + 2H^+ + 2ATP$$

Figure 3: Glycolysis Equation.

Concept 2.1: Overview of the regulatory reactions in glycolysis

- Key reactions that inhibit or promote glycolysis:

1. Phosphorylation of Glucose

- **Glucose → Glucose-6-phosphate**

2. Fructose 6-phosphate phosphorylation

- **Fructose-6-phosphate → Fructose-1,6 bisphosphate**

3. Pyruvate Formation

- **PEP → Pyruvate**

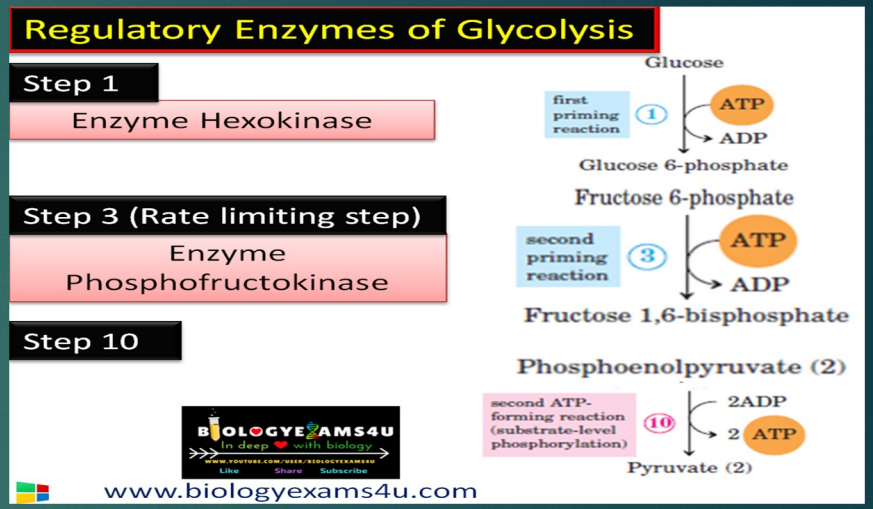


Figure 4: Overview of Regulatory Reactions.

Concept 2.2: Phosphorylation of Glucose

- Process:
  - Phosphate from ATP added to glucose by either hexokinase or glucokinase
    - Hexokinase (rest of cells):
      - Low Km (efficient phosphorylation) and Vmax (prevents overuse of glucose)
    - Glucokinase (liver/pancreas):
      - High Km (active at high glucose lvls) and Vmax (allows the liver to quickly remove glucose)
- Product:
  - Glucose 6-phosphate (G6P)
- Importance of Phosphorylation:
  - Traps glucose in cell, ensuring use in glycolysis
  - Later donated to ADP to form ATP
- Enzyme Regulation (Hexokinase/glucokinase):
  - Inhibited by G6P

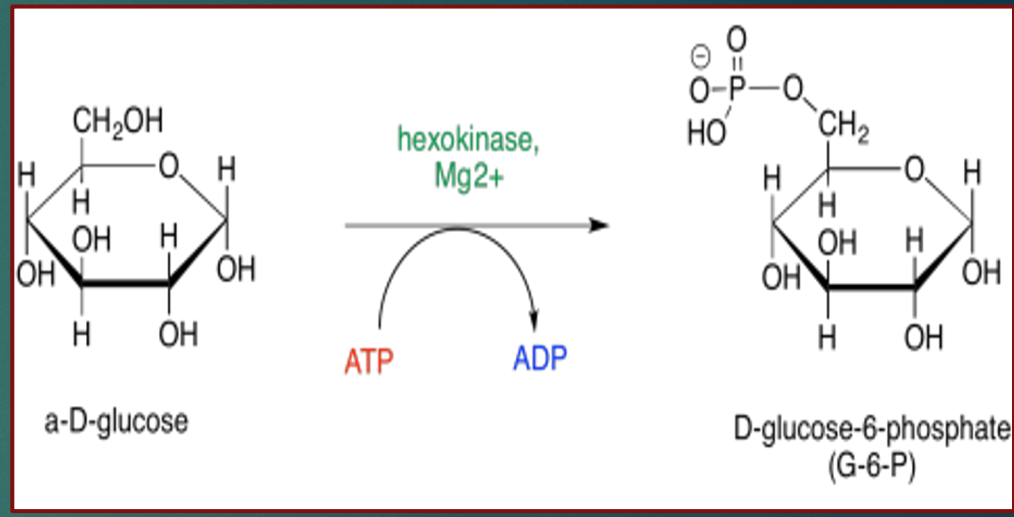


Figure 5: Phosphorylation of glucose.

Concept 2.3: Fructose-6-phosphate to Fructose-1,6-bisphosphate

- Process:
  - Phosphate from ATP added to fructose 6-phosphate by phosphofructokinase-1 (PFK-1)
- Product:
  - Fructose-1,6-bisphosphate
- Importance:
  - Rate committed step of glycolysis that ensures the process progresses
- Enzyme Regulation (PFK-1):
  - Activated by AMP allosterically (indicates lack of ATP/energy in cell)
  - Fructose 2,6-bisphosphate (most potent activator), generated by PFK-2 and elevated by insulin after a carb-rich meal
  - Inhibited by: ATP & citrate (heterotropic effector, indicates high energy state of cell)

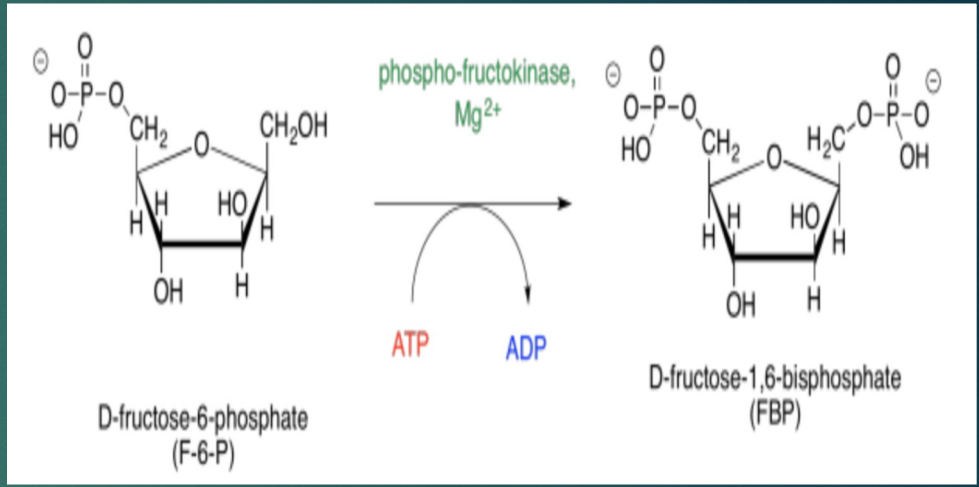


Figure 6: Phosphorylation of Fructose-1,6-bisphosphate.

Concept 2.4: PEP to pyruvate

- Process:
  - Phosphenolpyruvate has Inorganic Phosphate transferred by pyruvate kinase
- Product:
  - Pyruvate, ATP (x2)
- Importance:
  - Produces energy and the key substrate (pyruvate) for further metabolism
- Enzyme Regulation (Pyruvate kinase):
  - Activated by fructose 1,6- bisphosphate (product of PFK-1, ensures no buildup of glycolytic intermediates)

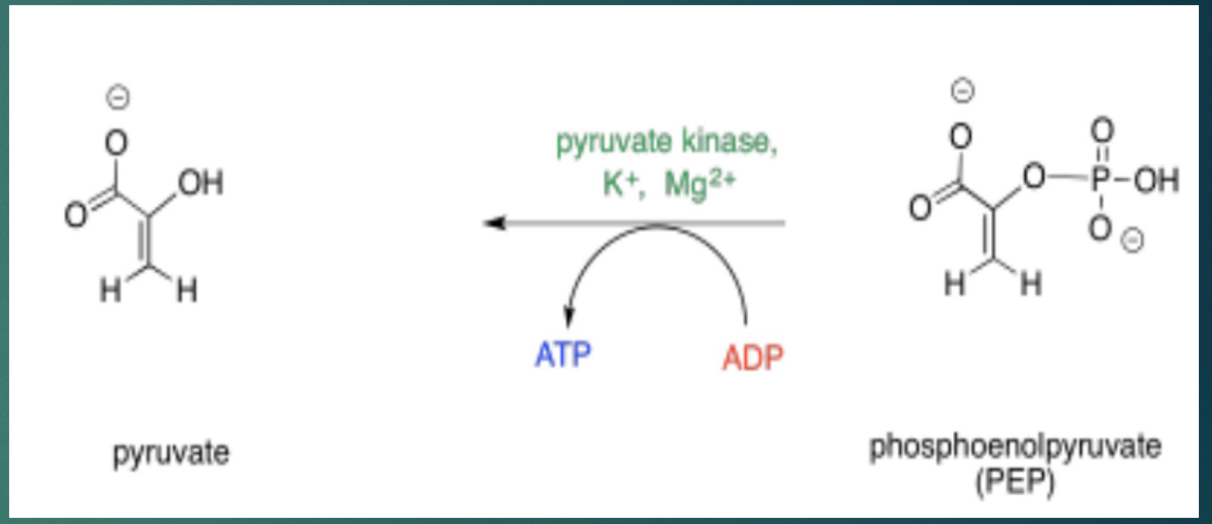


Figure 7: Production of Pyruvate.

Concept 2.5: Glycolysis Summary

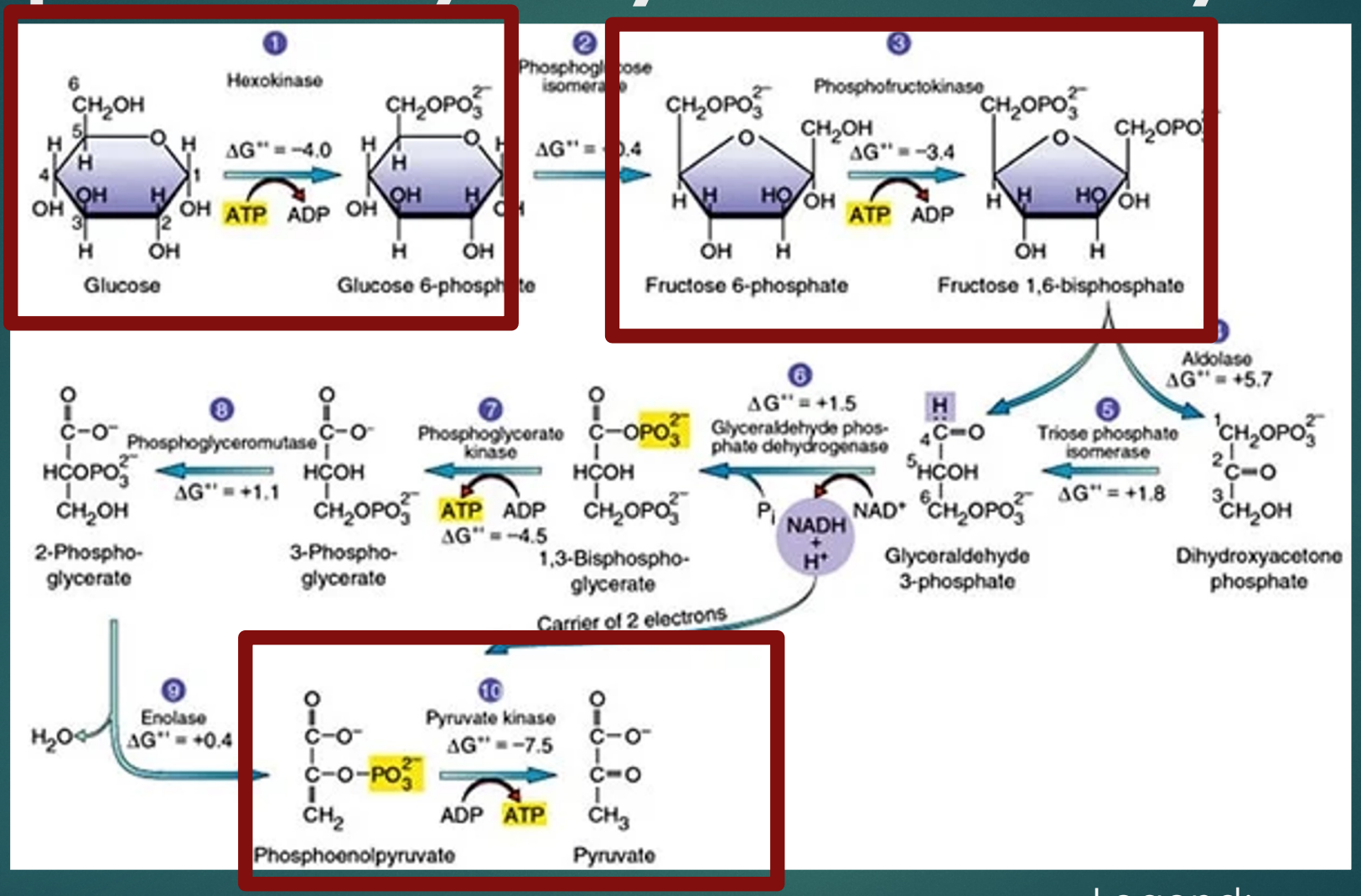


Figure 8: Glycolysis Pathway

Legend:

= Regulatory step

Section 2 Quiz

Q1. Which reaction is NOT a regulatory step of glycolysis?

A. Glucose → Glucose-6-phosphate
B. Fructose-6-phosphate → Fructose-1,6 bisphosphate
C. Glucose-6-phosphate → Fructose-6-phosphate
D. PEP → Pyruvate

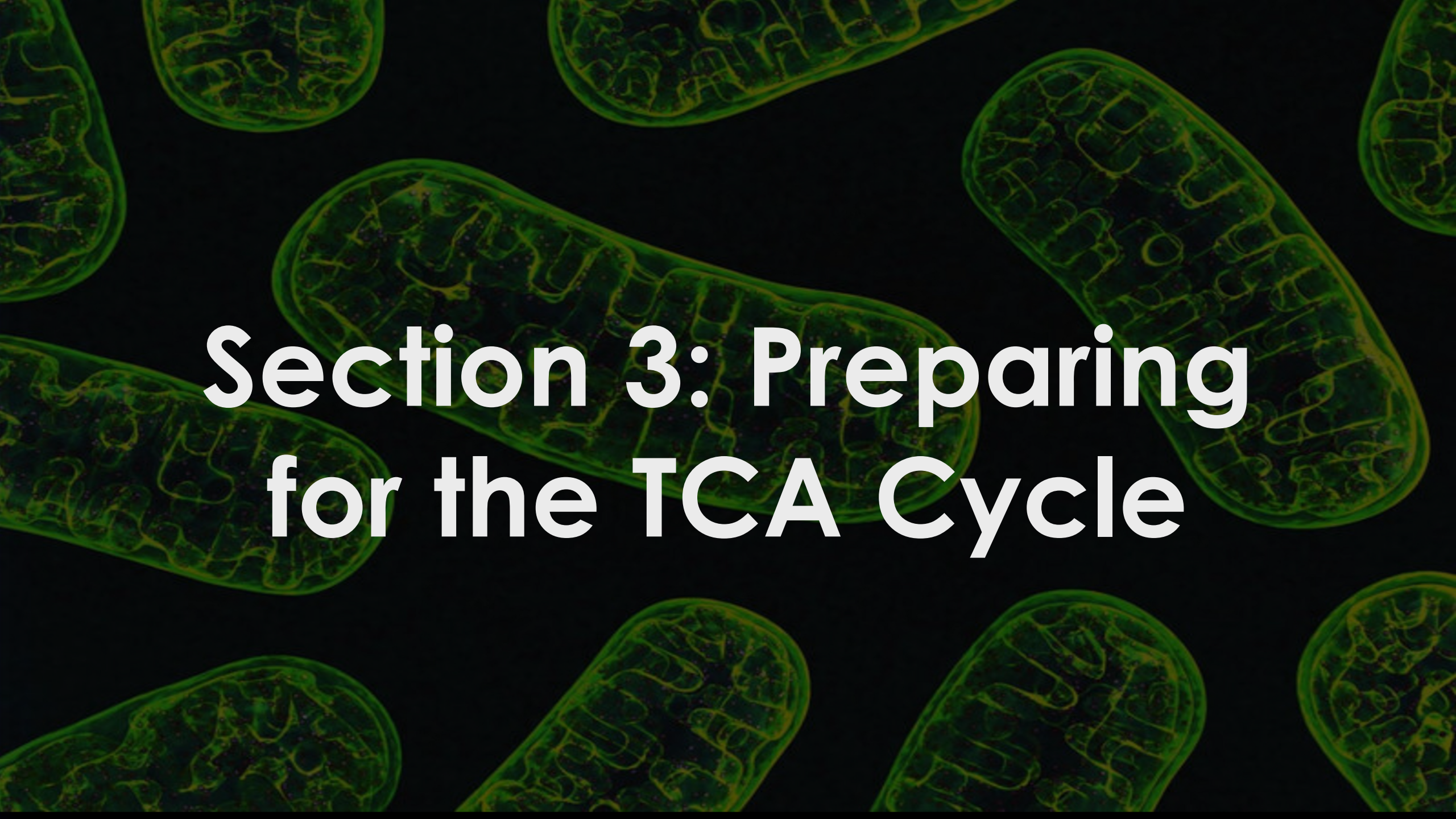
Answer: C

Section 2 Quiz

Q2.What are the enzymes that catalyze the phosphorylation of glucose? Explain where they are found and their differences in functionality.

Answer:

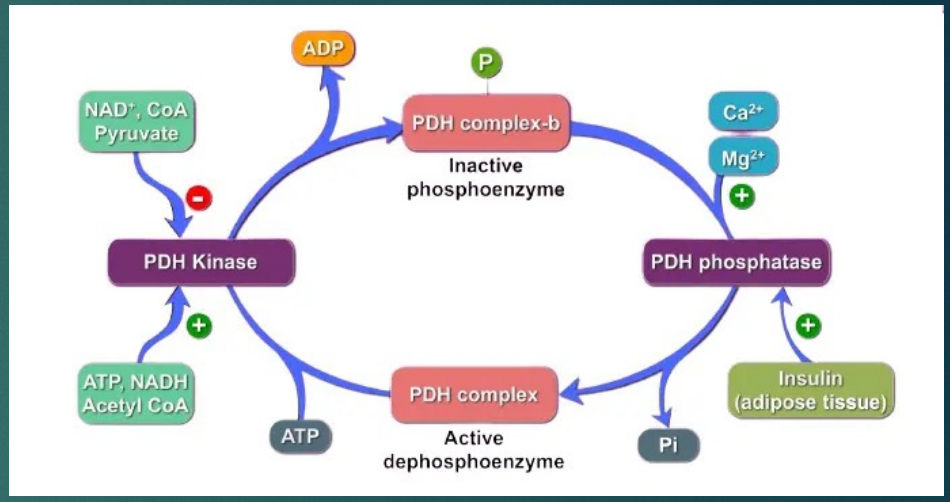
- Hexokinase (rest of cells):
  - Low Km (efficient phosphorylation) and Vmax (prevents overuse of glucose)
- Glucokinase (liver/pancreas):
  - High Km (active at high glucose lvls) and Vmax (allows the liver to quickly remove glucose)



Section 3: Preparing for the TCA Cycle

Concept 3.1: Conversion of pyruvate to acetyl-CoA

- Pyruvate dehydrogenase (PDH): converts pyruvate into acetyl-CoA.
  - The reaction occurs in the mitochondrial matrix
- PDH is regulated by several factors, including covalent modification and allosteric regulation.
  - PDH kinase phosphorylates and inactivates PDH, whereas PDH phosphatase dephosphorylates and activates it.
  - PDH kinase is activated by high levels of **ATP, NADH, and acetyl-CoA**
    - indicate that energy reserves are sufficient and that additional acetyl-CoA is not needed.
  - PDH phosphatase is activated by high levels of **ADP and pyruvate**
    - indicate that energy reserves are low and that additional acetyl-CoA is needed.
- Several factors act as activators or inhibitors of PDH.
  - Activators include pyruvate, CoA, and NAD+, which enhance the activity of the enzyme.
  - Inhibitors include acetyl-CoA, NADH, and ATP, which decrease the activity of the enzyme.



The different components that activate or inhibit the PDH complex.

Section 3 Quiz

Which of the following is an activator of Pyruvate dehydrogenase (PDH) enzyme?

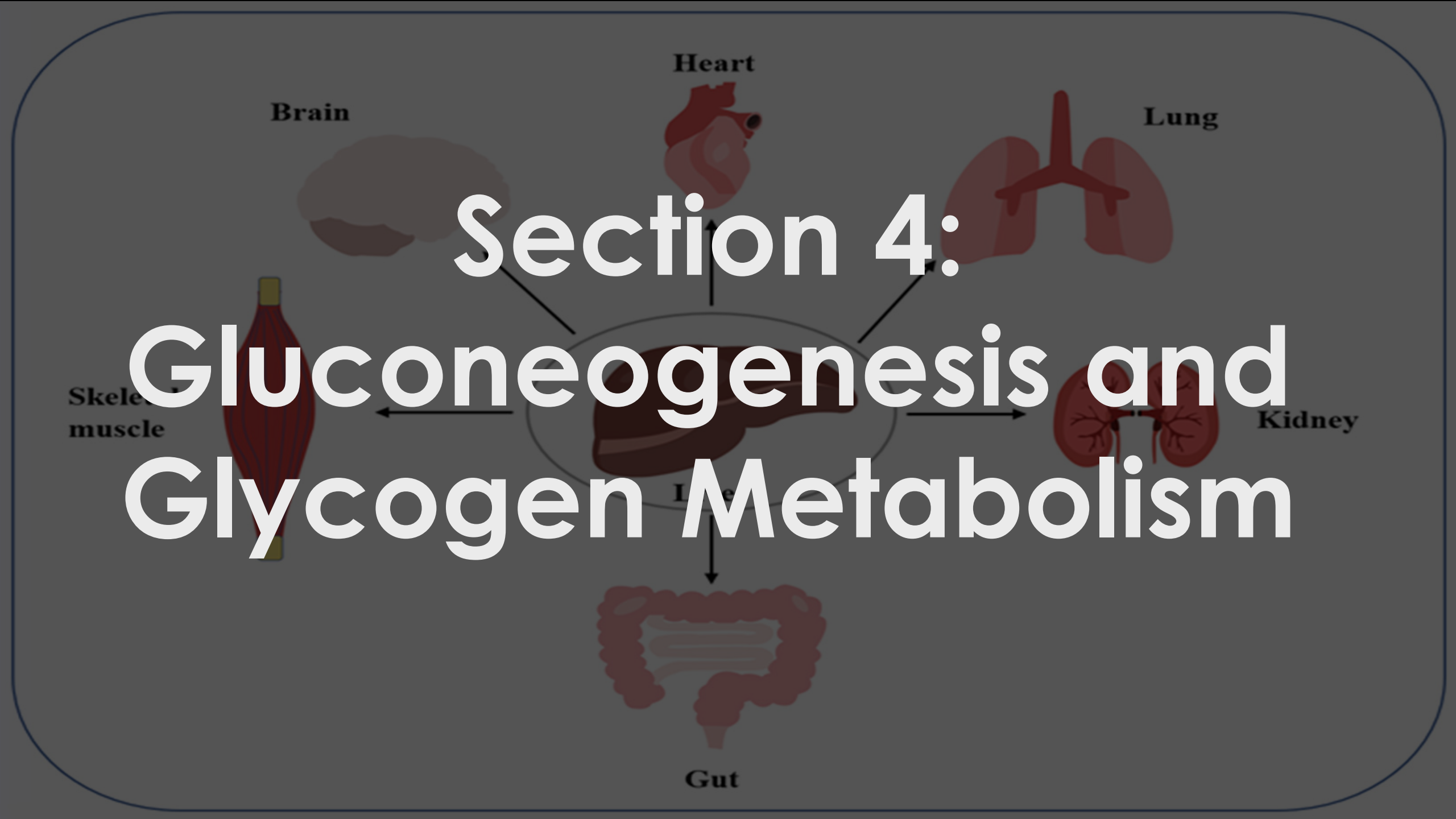
A. Acetyl-CoA

B. NADH

C. ATP

D. Pyruvate

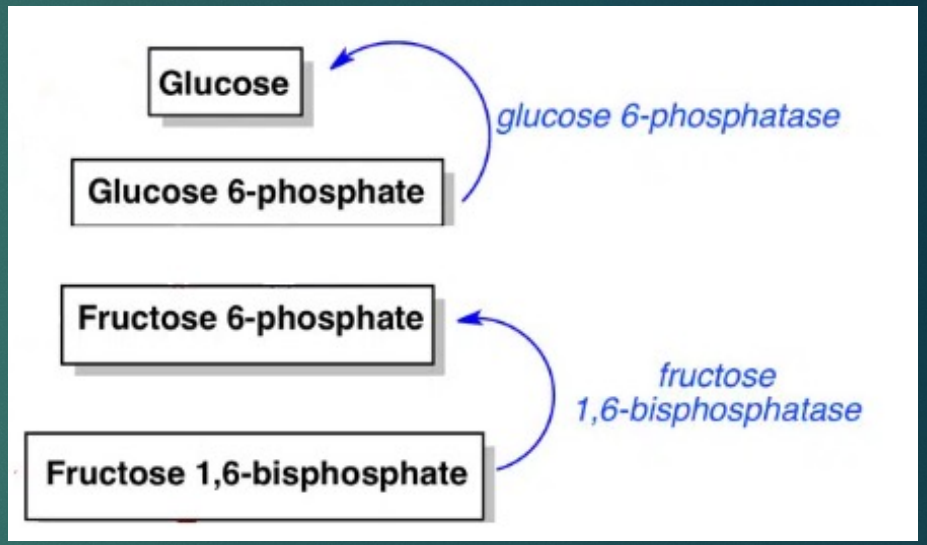
Correct answer: D. Pyruvate is an activator of the PDH enzyme, along with CoA and NAD+. Acetyl-CoA, NADH, and ATP are inhibitors of the enzyme.



Section 4: Gluconeogenesis and Glycogen Metabolism

Concept 4.1: Overview of Gluconeogenesis

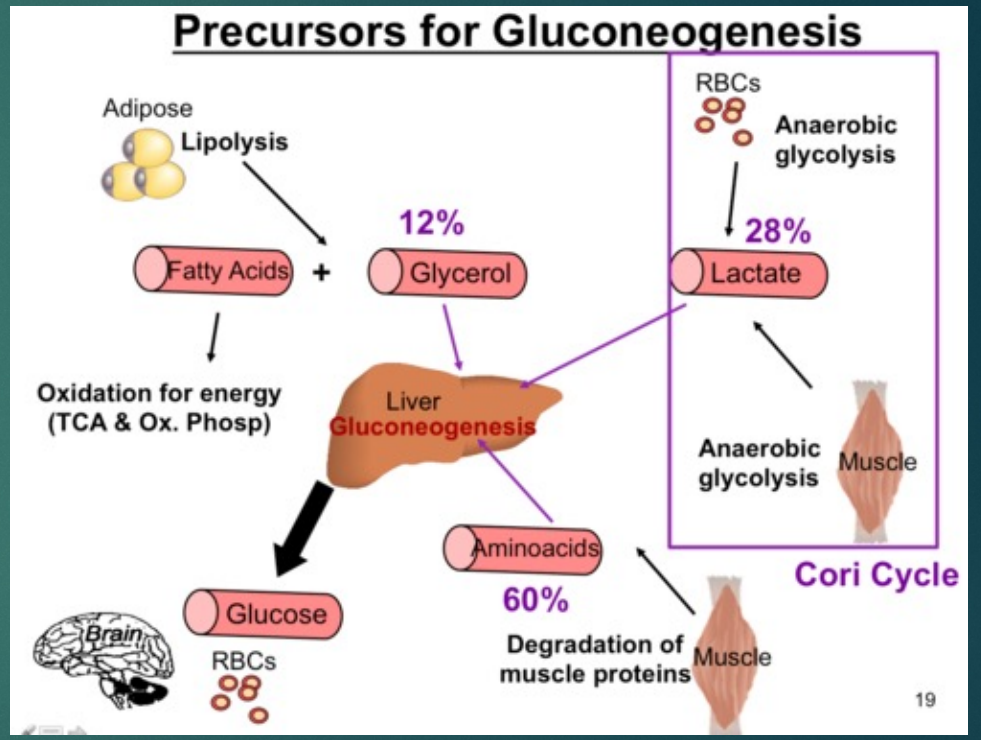
- Gluconeogenesis is a process that synthesizes glucose from non-carbohydrate sources when glucose levels are low.
- The liver and kidneys are the primary organs where gluconeogenesis occurs.
- Key regulatory reactions include the conversion of pyruvate to phosphoenolpyruvate and the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate.
- Pyruvate carboxylase is the enzyme involved in the conversion of pyruvate to PEP.
- Fructose-1,6-bisphosphatase catalyzes the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate.
- Other key enzymes involved in gluconeogenesis include glucose-6-phosphatase, which converts glucose-6-phosphate to glucose.



Simple Diagram showcasing the gluconeogenesis reaction.

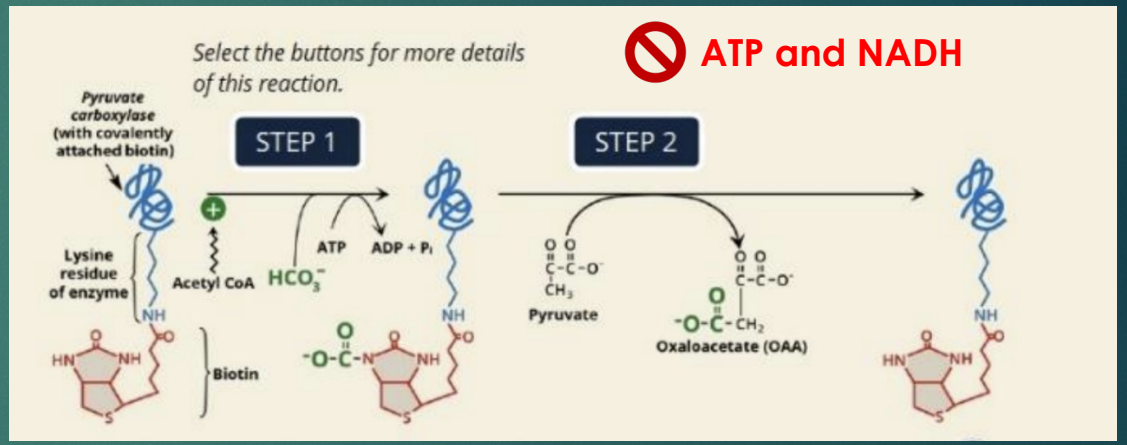
Concept 4.2: Diversity of Gluconeogenesis Substrates

- The substrates that can feed into gluconeogenesis include **glycerol, lactate, and amino acids.**
- Glycerol is derived from breakdown of triglycerides in adipose tissue
  - Converted to dihydroxyacetone phosphate (DHAP) in the liver.
- Lactate is produced by skeletal muscle during intense exercise
  - Transported to the liver, where it is converted to pyruvate and then to glucose via gluconeogenesis.
- Amino acids can also be used as substrates for gluconeogenesis.
  - Amino acids are converted to alpha-ketoglutarate, an intermediate of the citric acid cycle.
  - Alpha-ketoglutarate can then be used for gluconeogenesis.



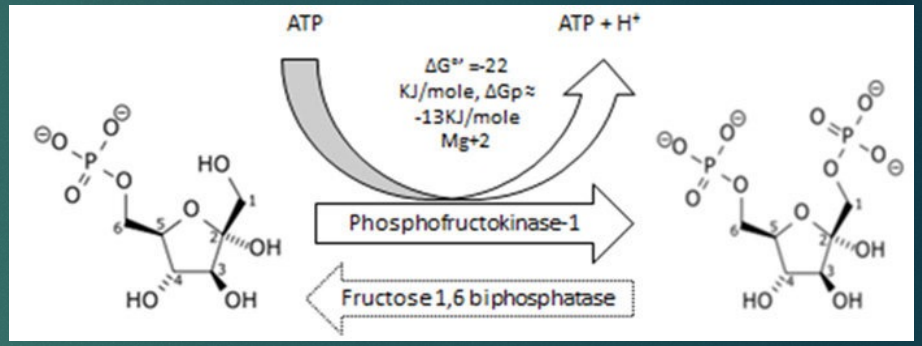
Concept 4.3: Carboxylation of Pyruvate

- Pyruvate carboxylase is an enzyme involved in the first committed step of gluconeogenesis, the carboxylation of pyruvate to oxaloacetate.
- The reaction occurs in the mitochondria of liver cells and is stimulated by the presence of acetyl-CoA, a high-energy molecule that indicates the availability of substrates for gluconeogenesis.
- The reaction proceeds in two steps:
  - first step involving the carboxylation of biotin, a coenzyme bound to the enzyme, with CO2 to form carboxybiotin.
  - In the second step, pyruvate reacts with carboxybiotin, forming oxaloacetate and releasing biotin.
- Pyruvate carboxylase is regulated by several factors, including covalent modification and allosteric regulation.
- Acetyl-CoA is a potent activator of pyruvate carboxylase, while ATP and NADH act as inhibitors.

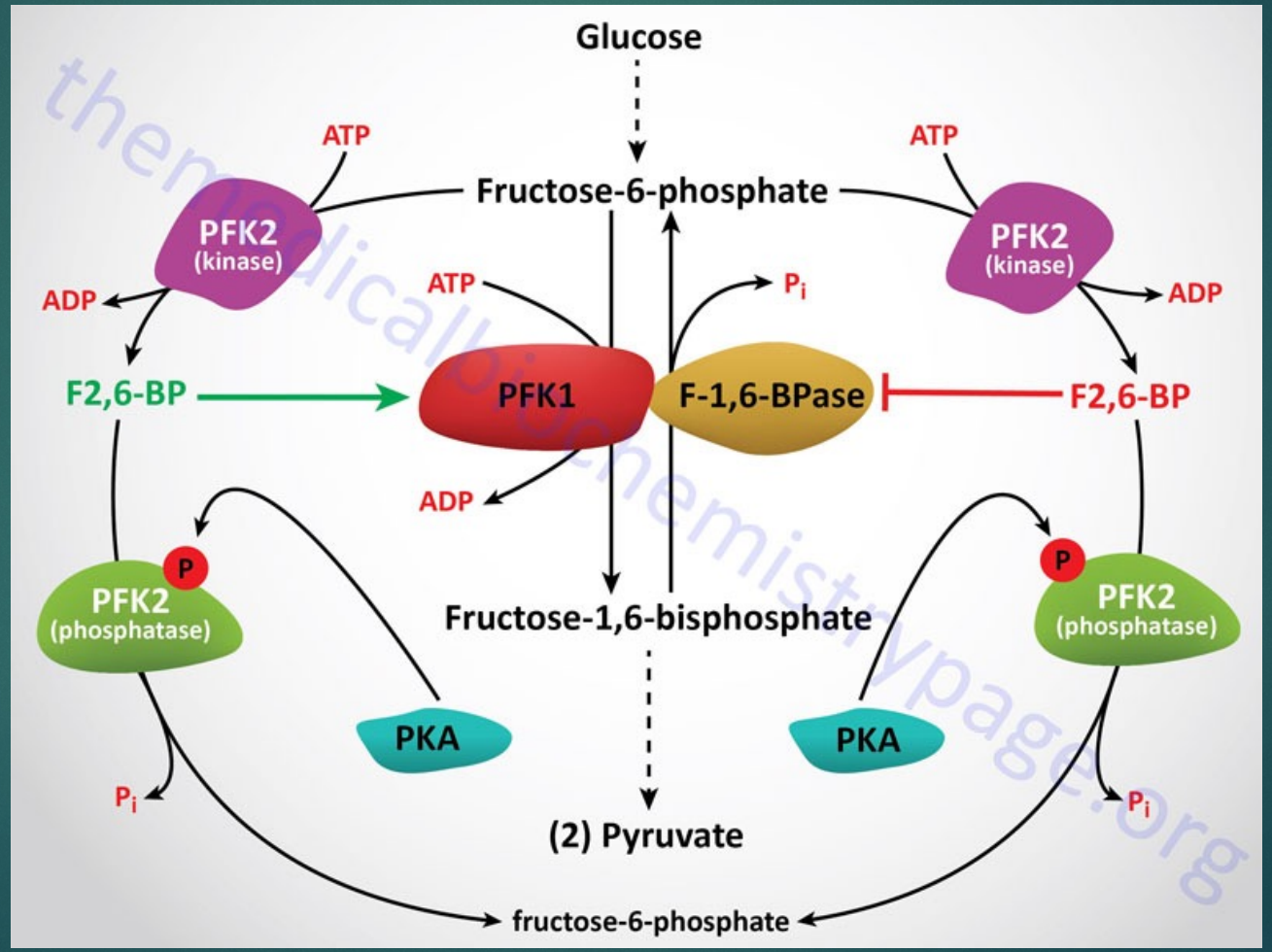


Concept 4.4: Dephosphorylation of Fructose 1,6-bisphosphate

- Fructose-1,6-bisphosphatase hydrolyses fructose-1,6-bisphosphate to produce fructose-6-phosphate and inorganic phosphate.
- This reaction is the reverse of phosphofructokinase-1 and helps regulate the flow of carbon through the pathway.
- The enzyme is regulated by covalent modification and allosteric regulation.
- **AMP and fructose-2,6-bisphosphate** are inhibitors, while **ATP and citrate** are **activators** of fructose-1,6-bisphosphatase.
- Protein kinase A and AMP-activated protein kinase can phosphorylate and inhibit the enzyme.
- Regulating fructose-1,6-bisphosphatase is important for maintaining glucose homeostasis and energy balance in the cell.

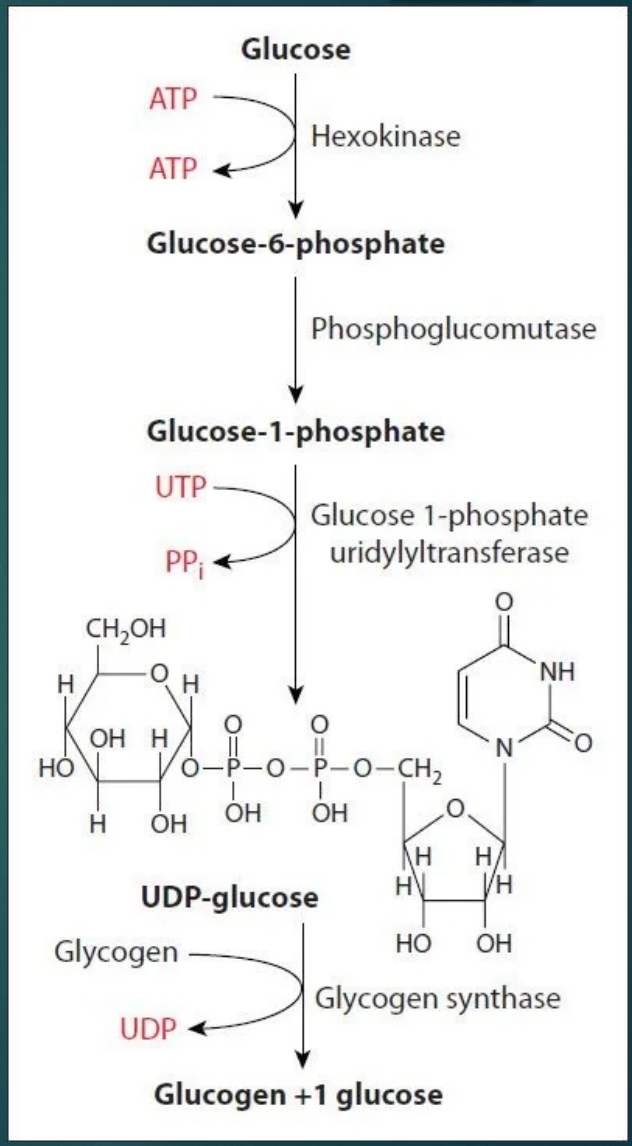


Concept 4.5: Gluconeogenesis Summary



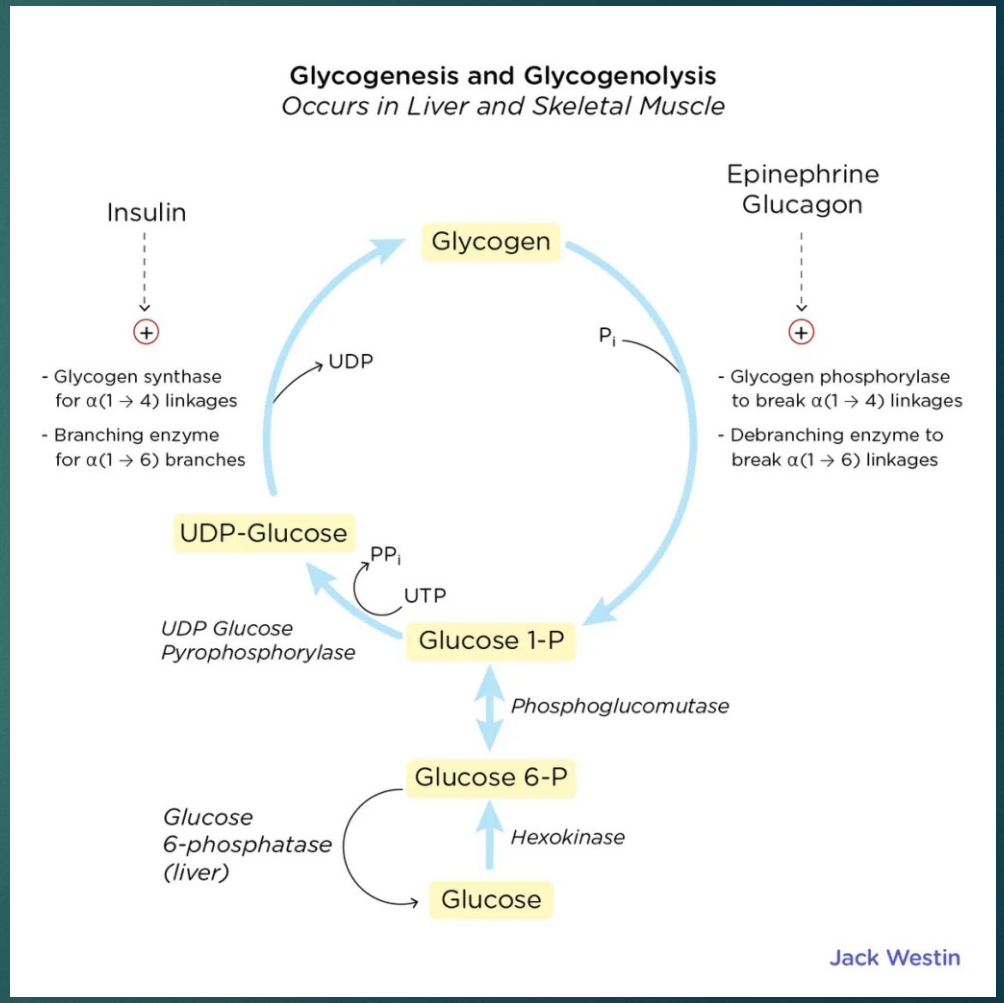
Concept 4.6: Glycogen Metabolism

- Regulation of Glycogen Synthase:
  - Hormonally regulated by insulin and glucagon
  - Insulin activates glycogen synthase by dephosphorylating it
  - Glucagon inhibits glycogen synthase by phosphorylating it
  - Allosterically activated by glucose-6-phosphate
  - Allosterically inhibited by ATP
- Regulation of Glycogen Phosphorylase:
  - Hormonally regulated by glucagon and epinephrine
  - Glucagon activates glycogen phosphorylase by phosphorylating it
  - Epinephrine activates glycogen phosphorylase by binding to its receptor and activating adenylate cyclase
  - Allosterically activated by AMP
  - Allosterically inhibited by ATP and glucose-6-phosphate



Concept 4.6: Glycogen Metabolism

- Glycolysis breaks down glucose into pyruvate and produces ATP, while gluconeogenesis synthesizes glucose from non-carbohydrate sources.
- Phosphofructokinase-1 is a key regulatory enzyme in both pathways that is activated by AMP and inhibited by ATP and citrate.
- Fructose-1,6-bisphosphatase is another regulatory enzyme that is inhibited by AMP and activated by ATP and citrate.
- Hormones like insulin promote glycolysis by activating PFK-1 and inhibiting FBPase, while glucagon promotes gluconeogenesis by inhibiting PFK-1 and activating FBPase.
- Factors such as pH, substrate availability, and gene expression also play a role in regulating these pathways.



Section 4 Quiz

What is the key regulatory enzyme involved in both glycolysis and gluconeogenesis?

A) Pyruvate carboxylase

B) Fructose-1,6-bisphosphatase

C) Phosphofructokinase-1

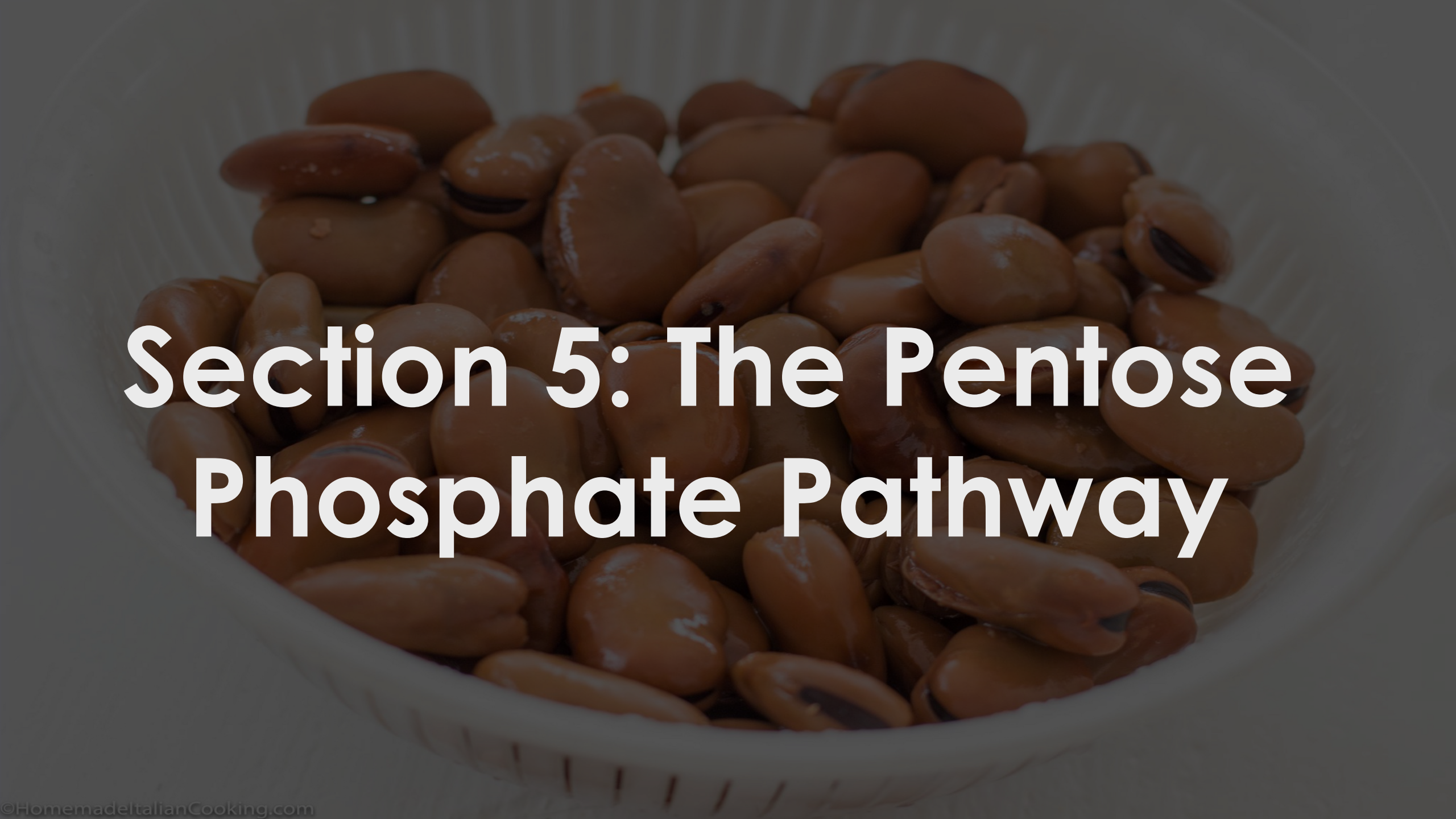
D) Glucose-6-phosphatase

Answer: C

Section 4 Quiz

Which hormone promotes glycolysis by activating phosphofructokinase-1 (PFK-1) and inhibiting fructose-1,6-bisphosphatase (FBPase)?

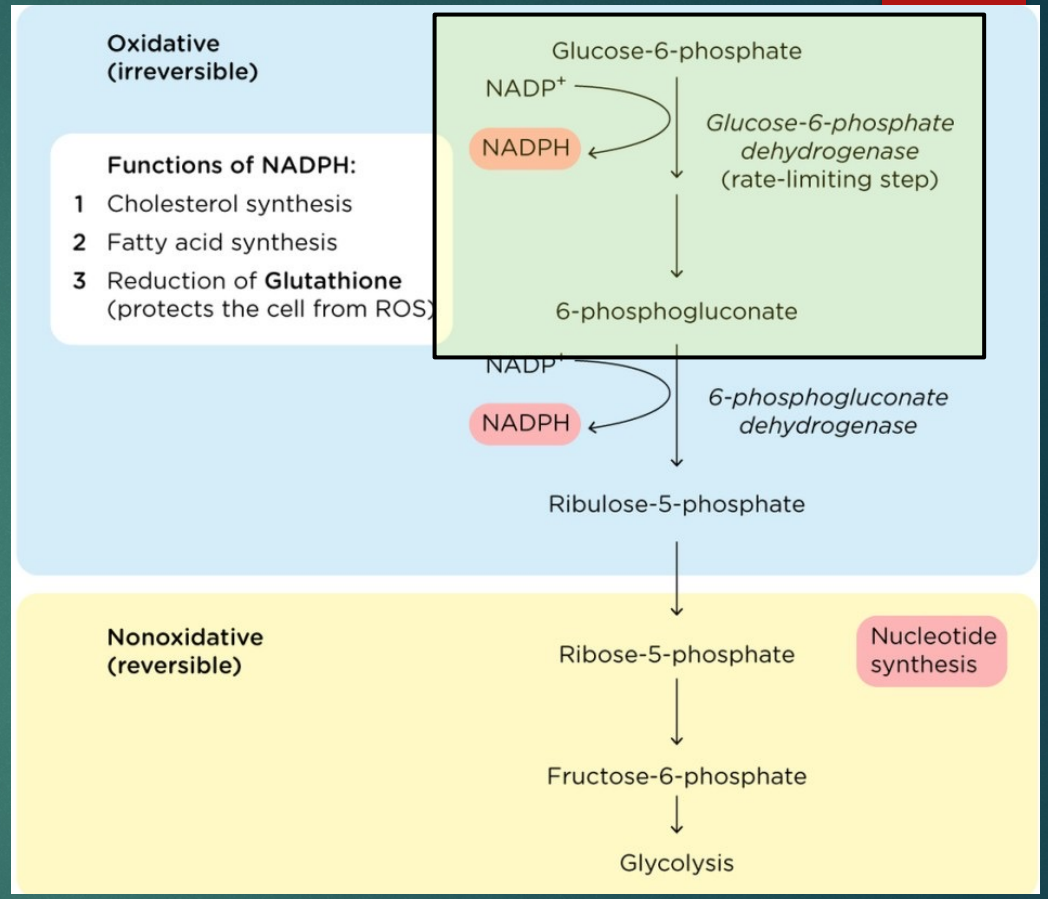
a) Insulin
b) Glucagon
c) Epinephrine
d) AMP



Section 5: The Pentose Phosphate Pathway

Overview of the Pentose Phosphate Pathway (PPP)

- **Location:** cytosol of liver, gonad, and red blood cells
- Consists of **two** irreversible oxidative reactions and a series of reversible non-oxidative reactions
- **No** ATP is consumed; main reactant: 1 **glucose-6-phosphate;** Main products: **1 CO_2** and **2 NADPH**



Oxidative and non-oxidative reactions of the PPP

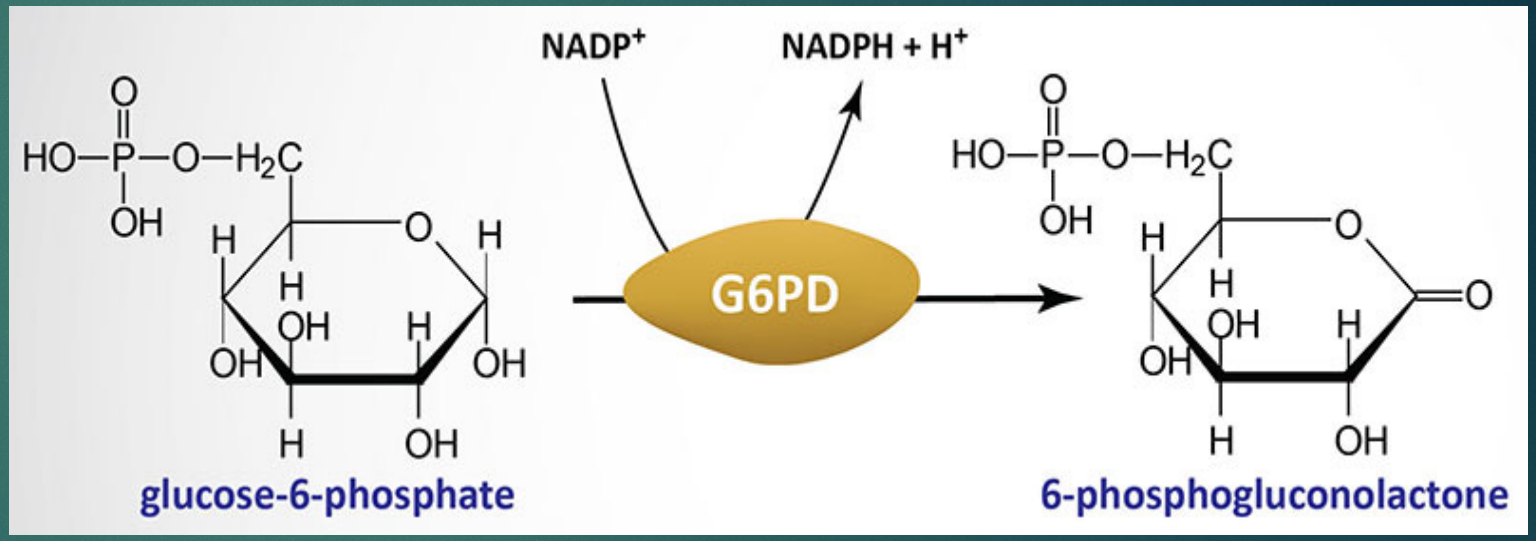
What to memorize about the PPP

- Location of pathway and cell types where it occurs
- Rate-limiting enzyme and its regulation (glucose-6-phosphate dehydrogenase (G6PD))
- Roles of NADPH
- Associated diseases (i.e., hemolytic anemia due to G6PD deficiency)

https://www.talktalkbnb.com/de/blog/article/why-is-it-so-hard-to-memorize-new-words/39

Dehydrogenation of glucose-6-phosphate: The Rate-Limiting Step

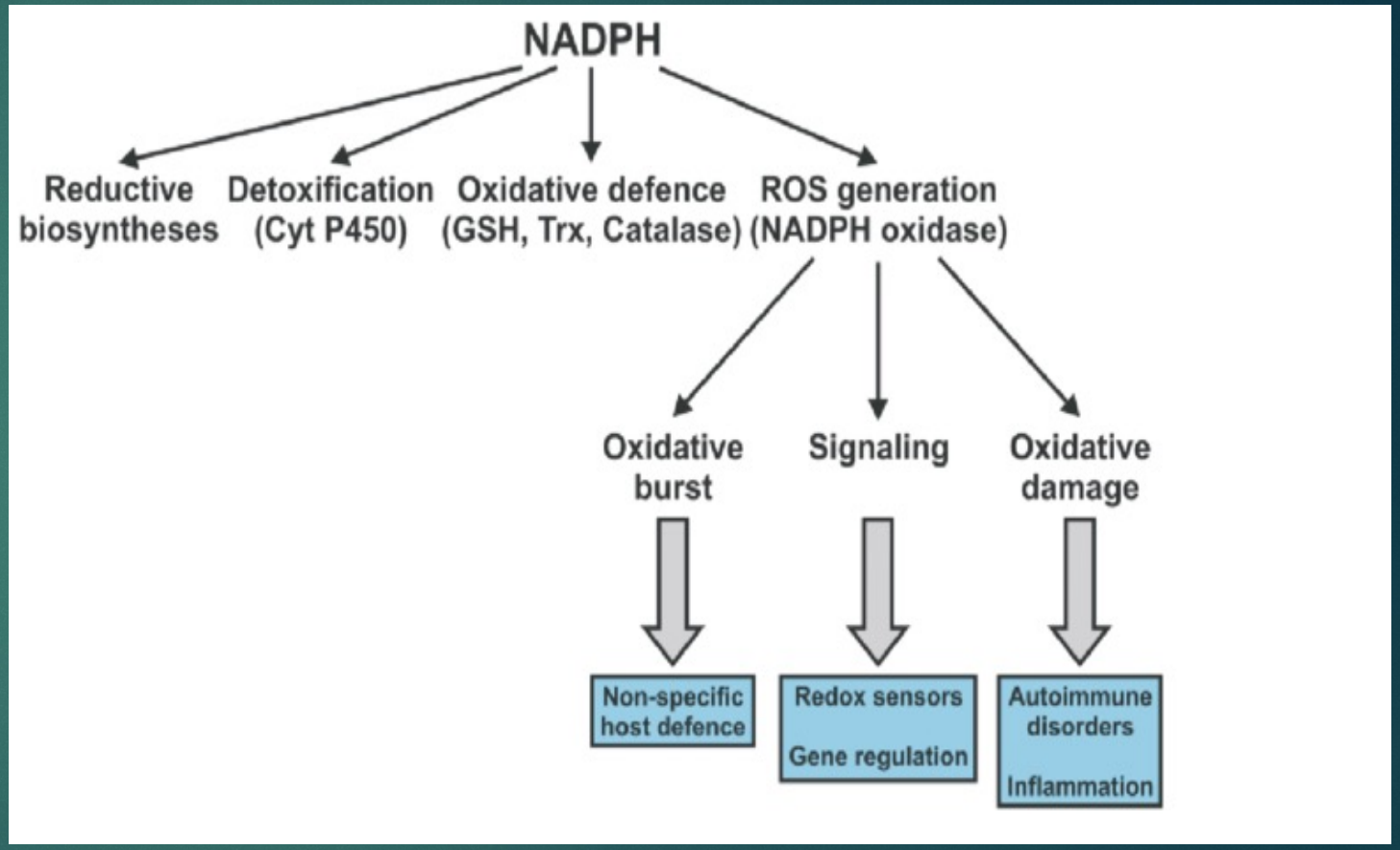
- G6PD is the **rate-determining enzyme** of the PPP and converts **glucose-6-phosphate** to **6-phosphogluconolactone**
- Produces first molecule of **NADPH**
- **Activator: insulin**
- **Inhibitor: NADPH (competitive inhibition)**



G6PD-catalyzed reaction

Importance of the PPP

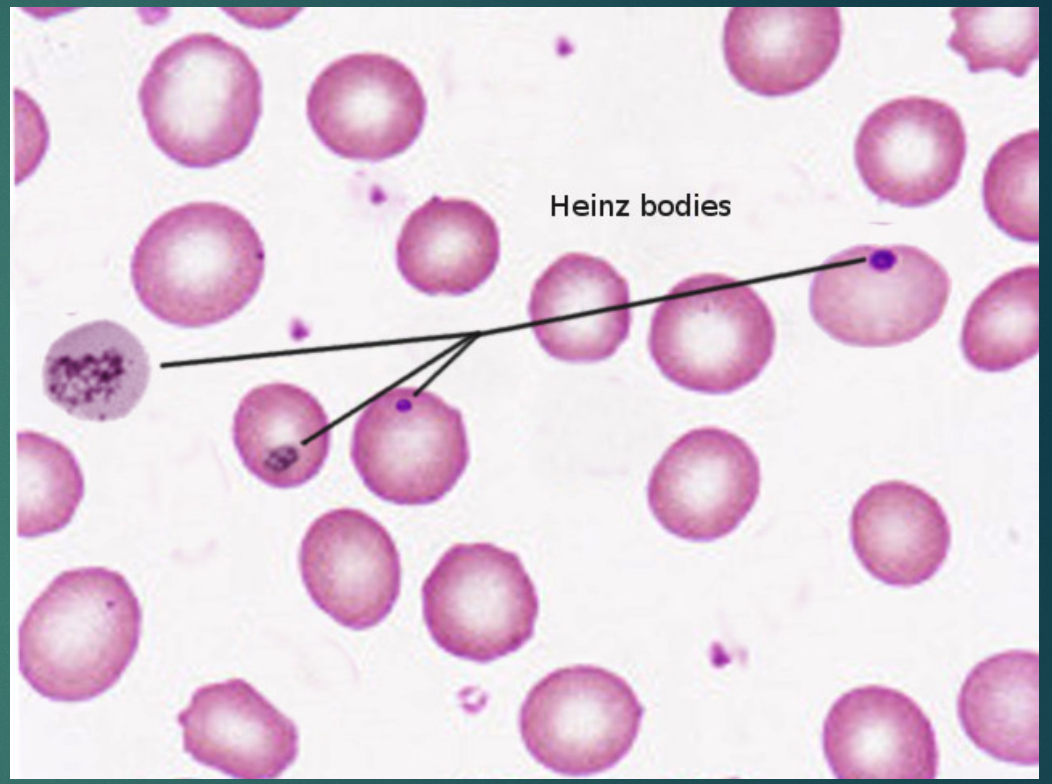
- Source of **NADPH**
- NADPH functions as a reductant in **biosynthetic** and **detoxification** reactions
- NADPH is needed for synthesis of **glutathione reductase** which detoxifies hydrogen peroxide
- NADPH is needed for the destruction of pathogens (e.g., bacteria and other microorganisms)



Functions of NADPH

Spotlight on Disease- G6PD Deficiency

- Hereditary disease characterized by **hemolytic anemia**
- Arises from an inability to detoxify oxidizing agents due to insufficient NADPH supply
- Low NADPH results in reduced levels of glutathione and in turn higher ROS
- ROS elevation leads to the formation of **'Heinz Bodies'** in red blood cells resulting in their premature removal from the circulation

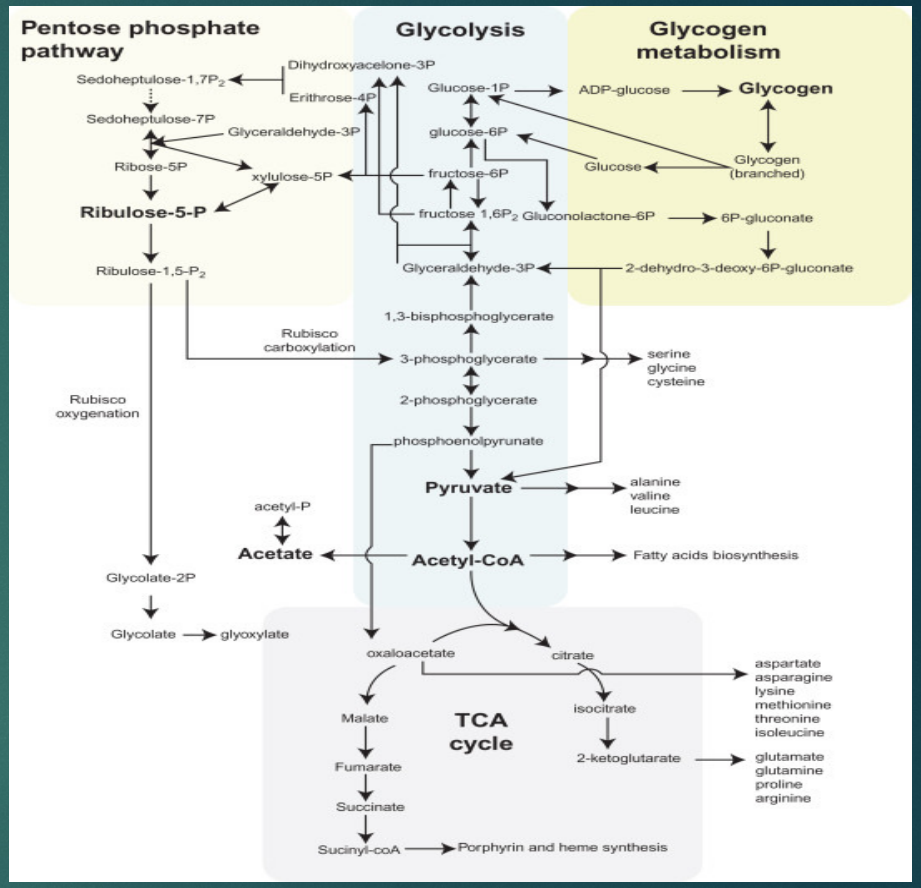


Heinz Bodies on red blood cells

PPP summary

Remember the following key points for your final exam:

- The PPP is a cytosolic pathway that converts **glucose-6-phosphate** to **ribose-5-phosphate**
- It produces the majority of **NADPH** required by the cell for **reductive biosynthesis** and **detoxification** reactions
- **G6PD** is the key regulatory enzyme of the pathway and is inhibited by **NADPH** (feedback inhibition) and leads to hemolytic anemia when deficient
- **Ribose-5-phosphate** is important for nucleotide biosynthesis and is converted to **glycolytic intermediates** when the cell does not require nucleotides



PPP intermediates can feed into glycolysis

Section 5 Quiz

Which of the following statements about the use of NADPH generated from the PPP is **not** correct?

a. It can be used for the regeneration of glutathione to its reduced state
b. It can be oxidized in the electron transport chain to produce ATP
c. It is used to support macrophagic functions
d. It is used for steroid synthesis

Answer: B