HNS 103: BIOCHEMISTRY

LECTURE 2: Carbohydrate Metabolism

01/03/2022

Dr. MULINGE
Email: mmulinge@uonbi.ac.ke

Course Outline

WEEK	DATE	TOPIC
1	22.02	BIOENERGETICS: standard free energy in chemical reactions, exergonic and endergonic reactions; Standard free energy of hydrolysis of ATP, Enzymatic transfer of phosphate groups to ATP; Properties of. ATP and high energy phosphate compounds
2	01.03	**CARBOHYDRATE METABOLISM:** Carbohydrate digestion & mobilization; Glycolysis and Its regulations, Substrate Level Phosphorylation; pyruvate oxidation.
3	08.03	**KREBS CYCLE:** Krebs cycle and regulation; Anaploretic reactions; phosphogluconate pathway.
4	15.03	**MITOCHODRIAL STRUCTURE & FUNCTION:** Electron Transfer Chain; Oxidative Phosphorylation; Mechanisms of ATP generation; Uncouplers; inhibitors of ATP generation
5	22.03	**DISACCHARIDE METABOLISM:** Phosphogluconate pathway; Glycogen metabolism; Glycogenolysis and gluconeogenesis; Regulation of glycogen metabolism; Covalent modification; cAMP and hormonal regulation; Glycogen storage disease
	29.03	• CAT I

Carbohydrate metabolism – a summary

- Dietary carbohydrates include starch, sucrose, lactose, and indigestible fiber.
- **Glucose is a major fuel source** that is oxidized by cells for energy. **After a meal, it is converted to glycogen or to triacylglycerols** and stored.
- Glucose is also converted to compounds such as **proteoglycans, glycoproteins, and glycolipids.**
- When glucose enters cells, it is converted to **glucose-6-phosphate,** which is a **pivotal compound in several metabolic pathways.**
- The major fate of glucose-6-phosphate is to enter the pathway of **glycolysis,** which produces pyruvate and generates NADH and ATP.
- **Glucose-6-phosphate** can be **converted to glucose-1-phosphate** and then to **UDP- glucose**, which is used for the **synthesis of glycogen or compounds such as the proteoglycans.**
- Glucose-6-phosphate can also enter the **pentose phosphate pathway**, which produces **NADPH** (for reactions such as the biosynthesis of fatty acids) and ribose-5-phosphate for nucleotide production.
- **Fructose and galactose** are converted to intermediates in the pathways by which glucose is metabolized.
- **Glycogen is the major storage form of carbohydrate in animals**. The largest stores are in muscle and liver.
- Muscle glycogen is used to generate ATP for muscle contraction.
- **Liver** glycogen is used to maintain blood glucose levels during fasting or exercise.
- The maintenance of blood glucose levels is a major function of the liver.
- The liver produces glucose by glycogenolysis and gluconeogenesis.

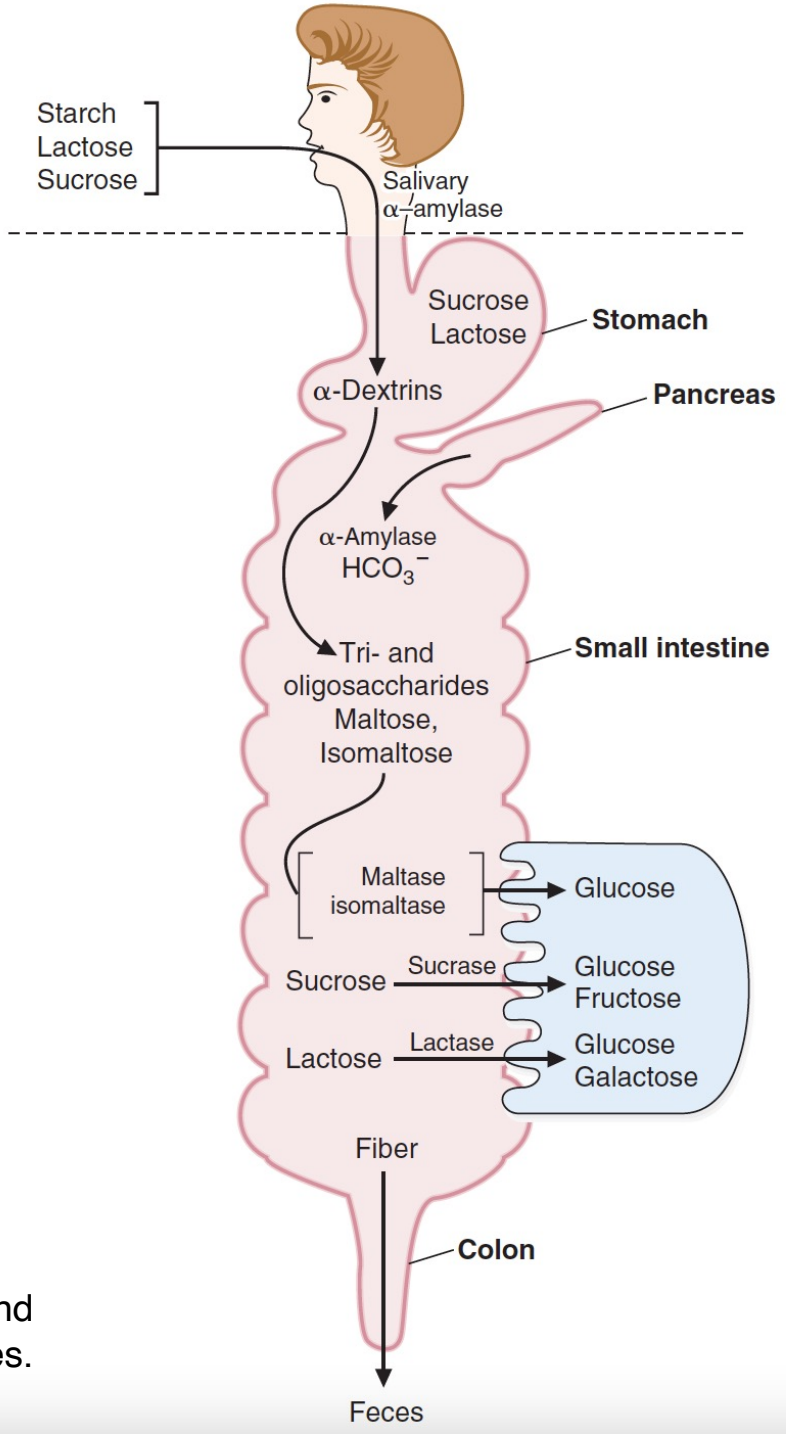
Carbohydrate Digestion

Dietary carbohydrates (mainly starch, sucrose, and lactose) constitute about 50% of the calories in the average diet (Western diets).

1. **Starch,** the storage form of carbohydrate in plants, is similar in structure to glycogen

Starch contains amylose (long, unbranched chains with glucose units linked α-1,4) and amylopectin (α-1,4-linked chains with α-1,6-linked branches). Amylopectin has fewer branches than glycogen.

2. **Sucrose** (a component of table sugar and fruit) contains glucose and fructose residues linked via their anomeric carbons.
3. **Lactose** (milk sugar) contains galactose linked β-1,4 to glucose



An overview of carbohydrate digestion. Starch is digested by salivary and pancreatic α-amylases and intestinal cell maltase and isomaltase. Sucrose and lactose are digested by intestinal enzymes. Subsequent metabolic reactions occur after the sugars are absorbed.

Carbohydrate Digestion - Mouth & intestines

Digestion of dietary carbohydrates in the mouth

In the mouth, salivary α-amylase cleaves starch by breaking α-1,4 linkages between glucose residues within the chains. Dextrins (linear and branched oligosaccharides) are the major products that enter the stomach.

Digestion of carbohydrates in the intestine

The stomach contents pass into the intestine where the bicarbonate secreted by the pancreas neutralizes the stomach acid, raising the pH into the optimal range for the action of the intestinal enzymes.

1. Digestion by pancreatic enzymes

a. The pancreas secretes an α-amylase that acts in the lumen of the small intestine and, like salivary amylase, cleaves α-1,4 linkages between glucose residues.

b. The products of pancreatic α-amylase are the disaccharides maltose and isomaltase, trisaccharides, and small oligosaccharides containing α-1,4 and α-1,6 linkages.

2. Digestion by enzymes of intestinal cells

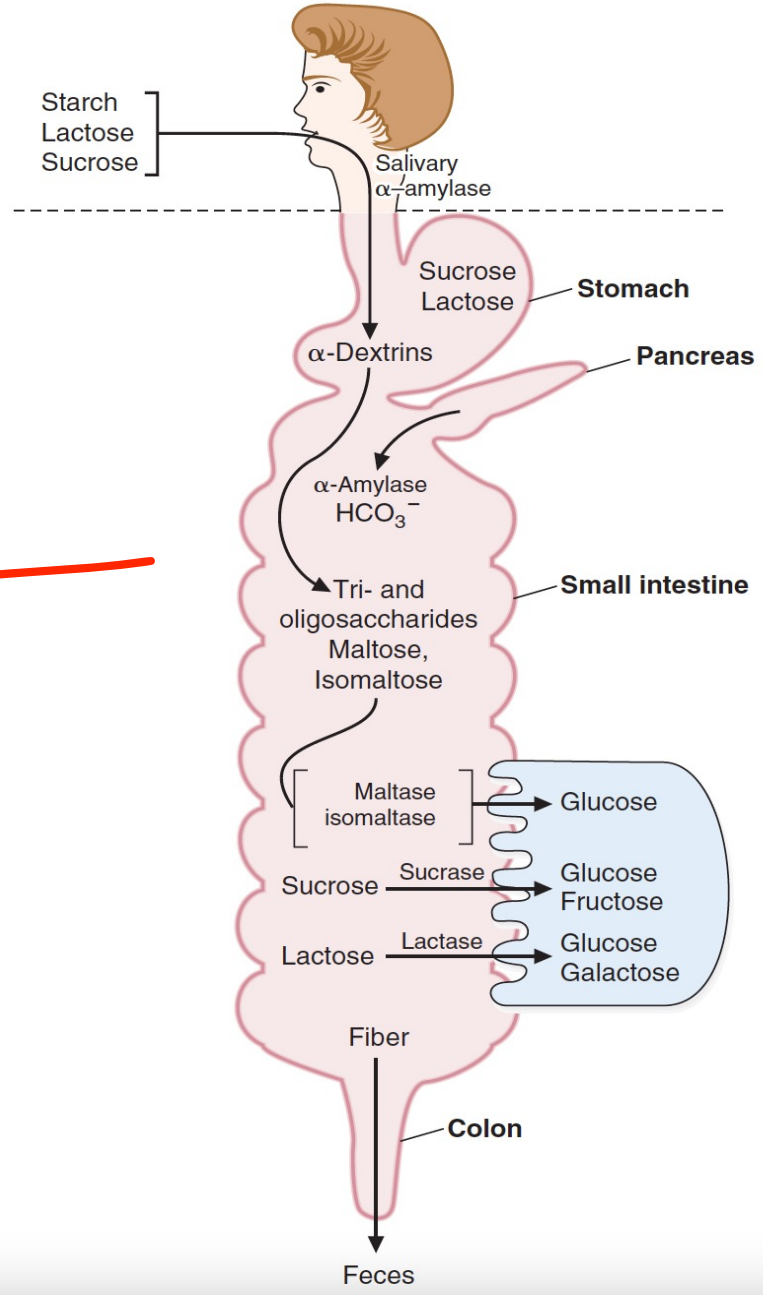
a. Complexes of enzymes, produced by intestinal epithelial cells and located in their brush borders, continue the digestion of carbohydrates

(1) Glucoamylase (an α-glucosidase) and other maltases cleave glucose residues from the nonreducing ends of oligosaccharides and also cleave the α-1,4 bond of maltose, releasing the two glucose residues.

(2) Isomaltase cleaves α-1,6 linkages, releasing glucose residues from branched oligosaccharides.

(3) Sucrase converts sucrose to glucose and fructose.

(4) Lactase (a β-galactosidase) converts lactose to glucose and galactose.

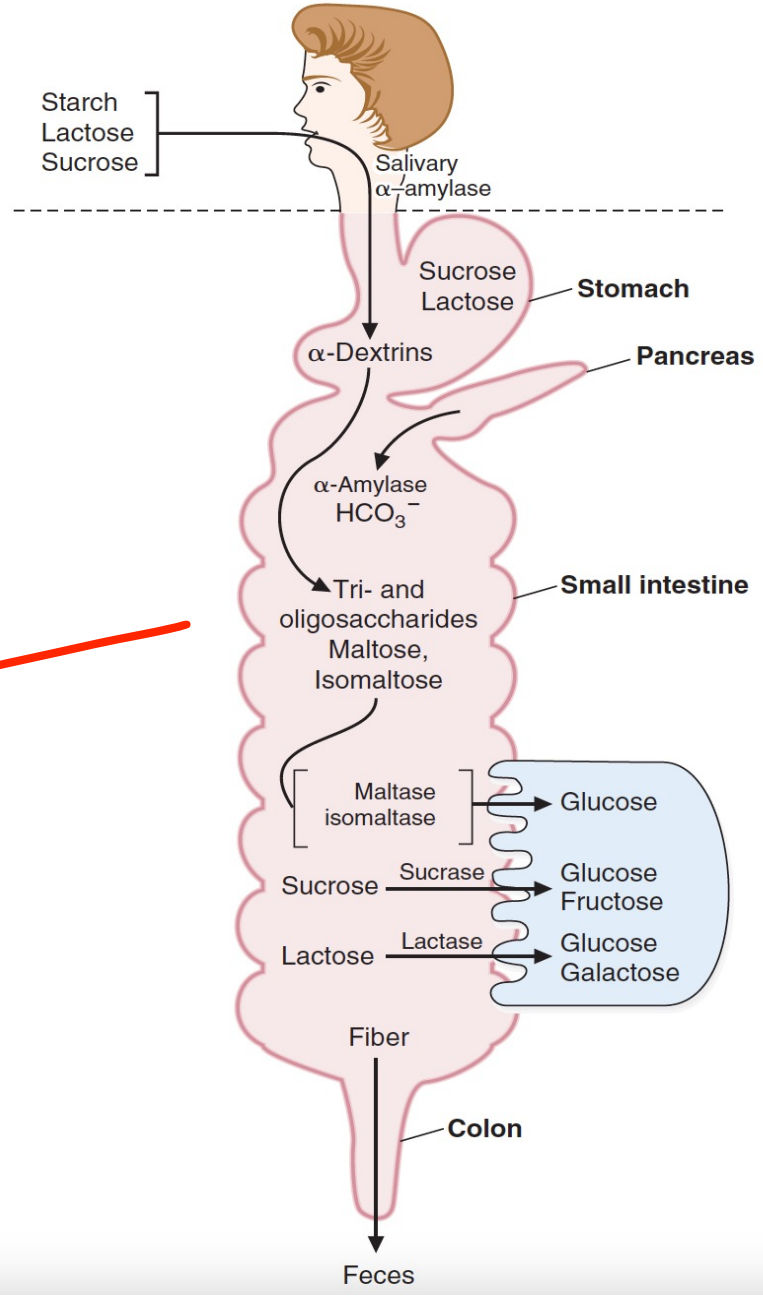


Carbohydrate Digestion - Colon

D. Carbohydrates that cannot be digested

Indigestible polysaccharides are part of the dietary fiber that passes through the intestine into the colon. For example, because enzymes produced by human cells cannot cleave the β-1,4 bonds of cellulose, this polysaccharide is indigestible.

In the colon they are digested by the microbiome (symbiotic bacteria) to produce metabolites including vitamins.



Clinical correlates

CLINICAL CORRELATES

Intestinal lactase deficiency is a common condition in which **lactose cannot be digested** and is oxidized by bacteria in the gut, which produce gas, and cause bloating and watery diarrhea. This can also occur through a loss of intestinal epithelial cells due to viral gastroenteritis.

GLYCOLYSIS Greek: glycos (sugar) and lysis (dissolution)

HISTORY OF GLYCOLYSIS

1897 – Hans Buchner & Edward Buchner:

● Sucrose added to cell-free extracts of yeast results in the formation of alcohol. ➔ Fermentation can occur outside living cells.

1905 – Arthur Harden & William Young:

● Glucose added to yeast extract results in an immediate start of the fermentation, but the rate of fermentation rapidly decreases unless inorganic phosphate is added

●Inorganic phosphate is incorporated into sugar phosphates

● Yeast juice contains at least 2 kinds of substances necessary for fermentation, i.e. “zymase” and “cozymase”.

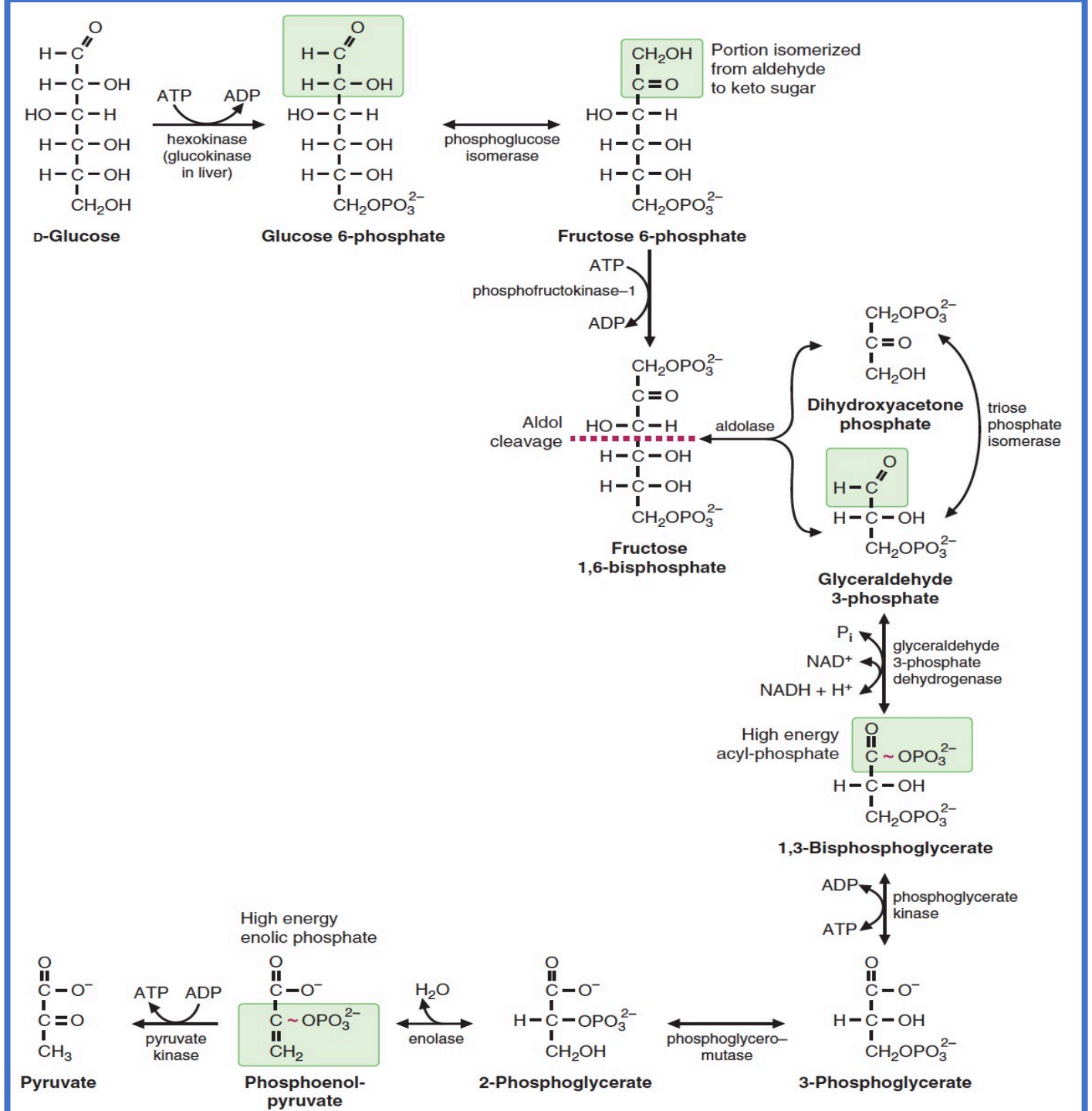
Today: zymases are enzymes, cozymases are metal ions, ATP, ADP, coenzymes (NAD, FAD, ...), magnesium, ...

1940 – Gustav Embden, Otto Mayerhof, Otto Warburg, Carl Neuberg, Jacob Parnas, and Gerty & Carl Cori: elucidation of the glycolytic pathway

GLYCOLYSIS

KEY CONCEPTS

- Glycolysis is a 10-step pathway in which glucose is converted to two molecules of pyruvate.
- Energy is invested in the first half of the pathway, and the second half of the pathway generates 2 ATP and 2 NADH.
- Flux through the pathway is controlled primarily at the phosphofructokinase step.
- Pyruvate can be converted to lactate, acetyl-CoA, or oxaloacetate.

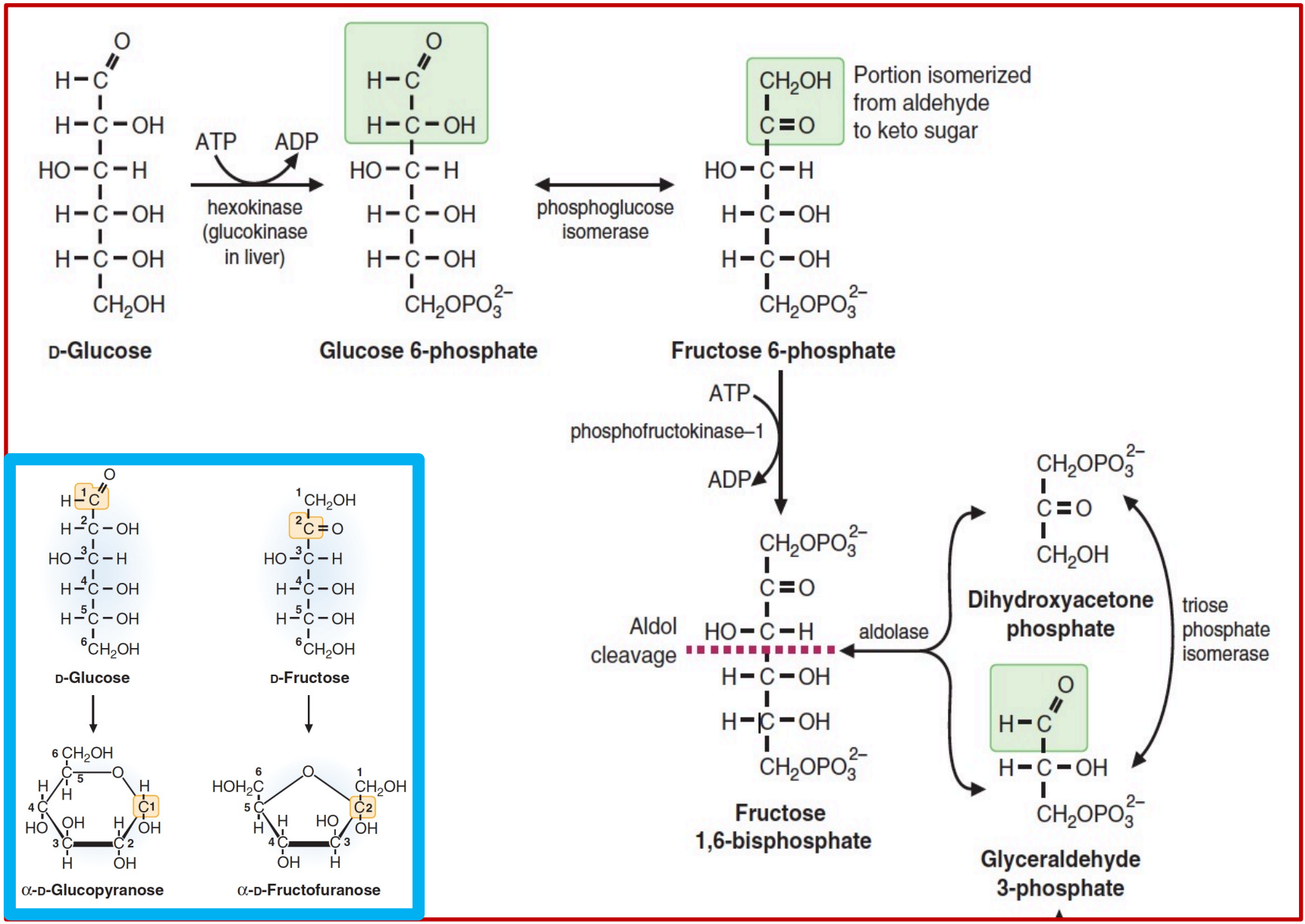


GLYCOLYSIS: Input phase

Glycolysis is the pathway by which glucose is converted to pyruvate. It occurs in the cytosol of all cells of the body.

- **In the initial reactions**, a hexose is phosphorylated twice by ATP and then cleaved to yield two triose phosphates.
- Glucose is phosphorylated to glucose-6-phosphate, which is **isomerized** to fructose-6-phosphate.
- Fructose-6-phosphate is **phosphorylated** by the key regulatory enzyme, **phosphofructokinase-1** to form **fructose-1,6-bisphosphate**, which is cleaved, forming two triose phosphates.

In the initial reactions (1st half):

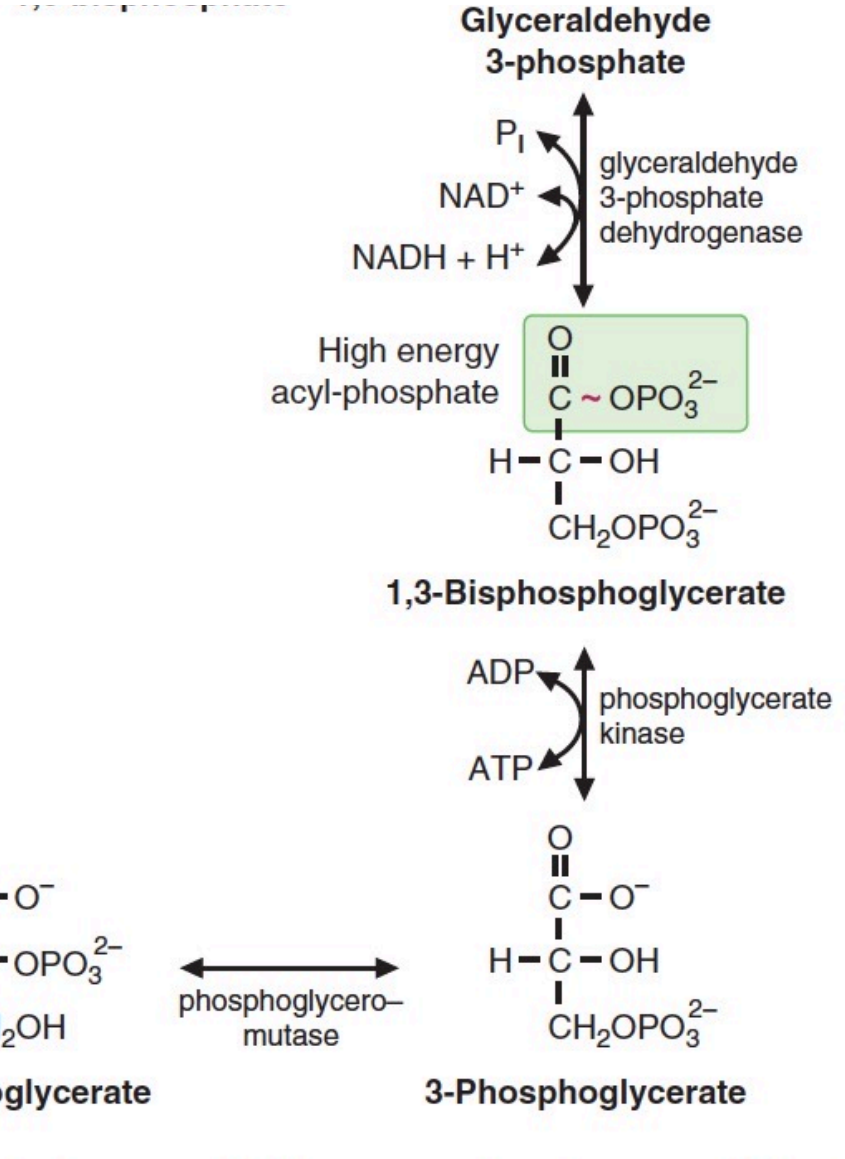


GLYCOLYSIS: harvest phase

In the second phase of reactions, the triose phosphates produce ATP.

- Overall, glycolysis produces ATP, NADH, and pyruvate.
- **ATP is produced directly** by reactions catalyzed by phosphoglycerate kinase and pyruvate kinase.
- Although **NADH** produced in the cytosol cannot directly enter mitochondria, reducing equivalents can be shuttled into this organelle, where they generate ATP.
- Pyruvate can enter mitochondria and be converted to acetyl-CoA, which is oxidized by the tricarboxylic acid (TCA) cycle, generating additional ATP.
- Pyruvate can also be converted to oxaloacetate (OAA) by a reaction that replenishes the intermediates of the TCA cycle.

second phase: pay-off phase



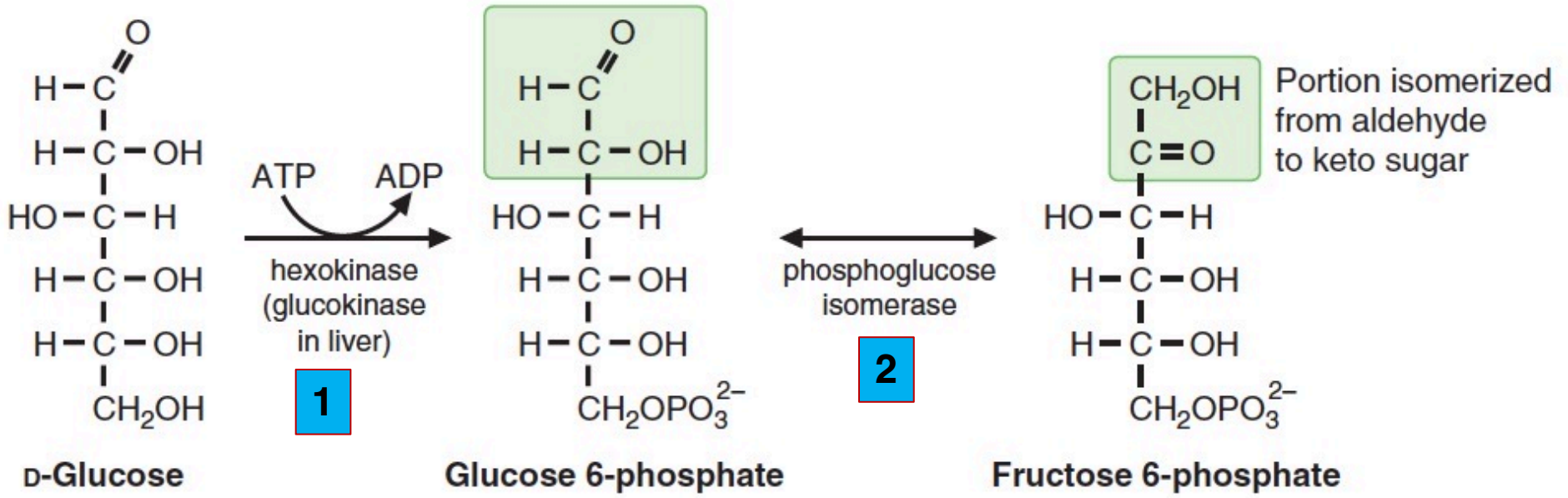
Reactions in Glycolysis

Step 1: Glucose is converted to glucose-6-phosphate in a reaction that uses ATP and produces ADP.

Enzymes: hexokinase in all tissues and, in the liver and pancreas, glucokinase. Both of these enzymes are subject to regulatory mechanisms.

Step 2. Glucose-6-phosphate is isomerized to fructose-6-phosphate.

a. Enzyme: phosphoglucose isomerase

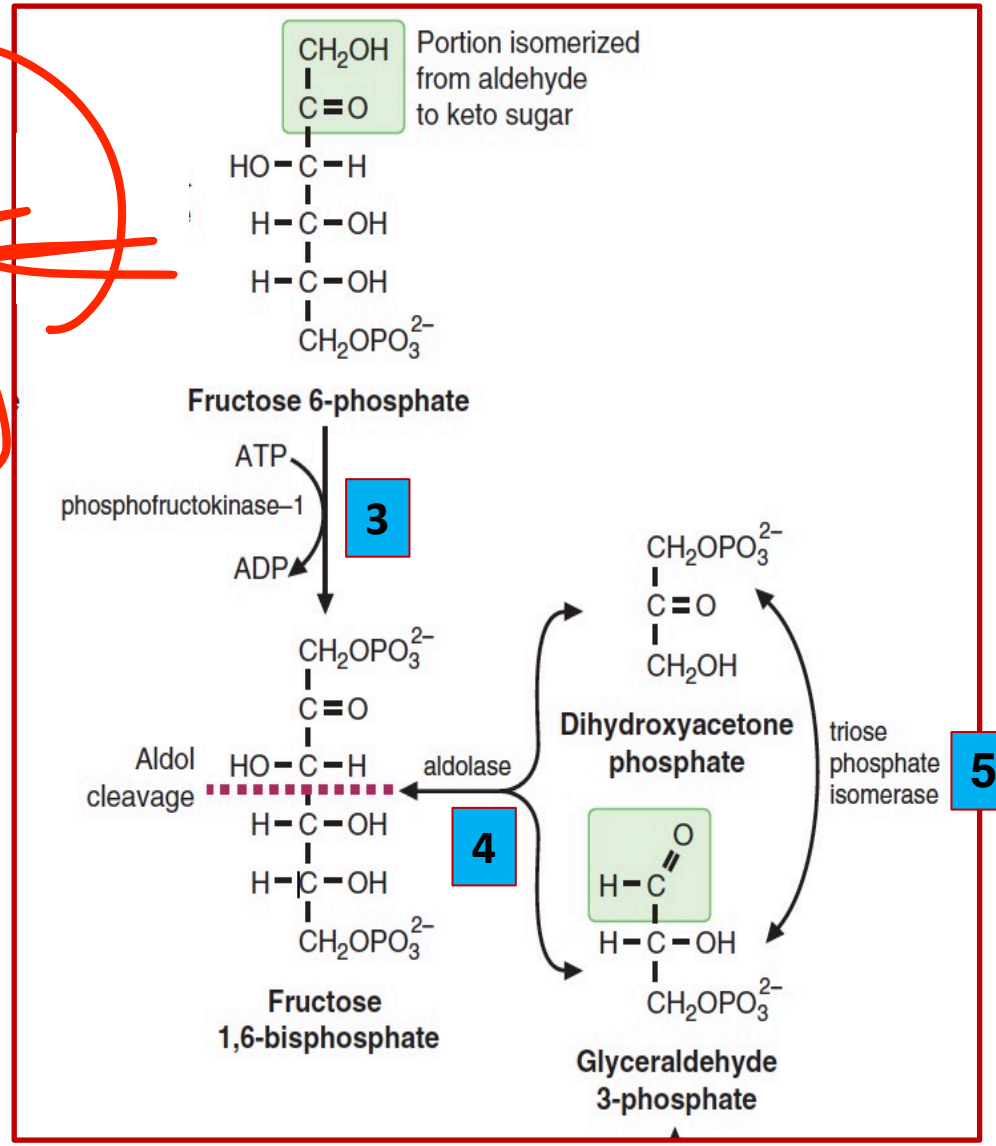


Reactions in Glycolysis

Step 3: Fructose-6-phosphate is **phosphorylated** by ATP, forming **fructose-1,6-bisphosphate** and ADP.
This reaction is the first committed step in glycolysis.
a. **Enzyme: phosphofructokinase 1 (PFK1)**

Step 4: Fructose-1,6-bisphosphate is **cleaved** to form the triose phosphates, glyceraldehyde-3-phosphate & dihydroxyacetone phosphate (DHAP).
a. Enzyme: aldolase

Step 5: DHAP is isomerized to glyceraldehyde-3-phosphate.
Enzyme: triose phosphate isomerase
(1) Note: The net result of reactions 1 through 5 is that 2 moles of glyceraldehyde-3- phosphate are formed from 1 mole of glucose, at the expense of two ATPs.



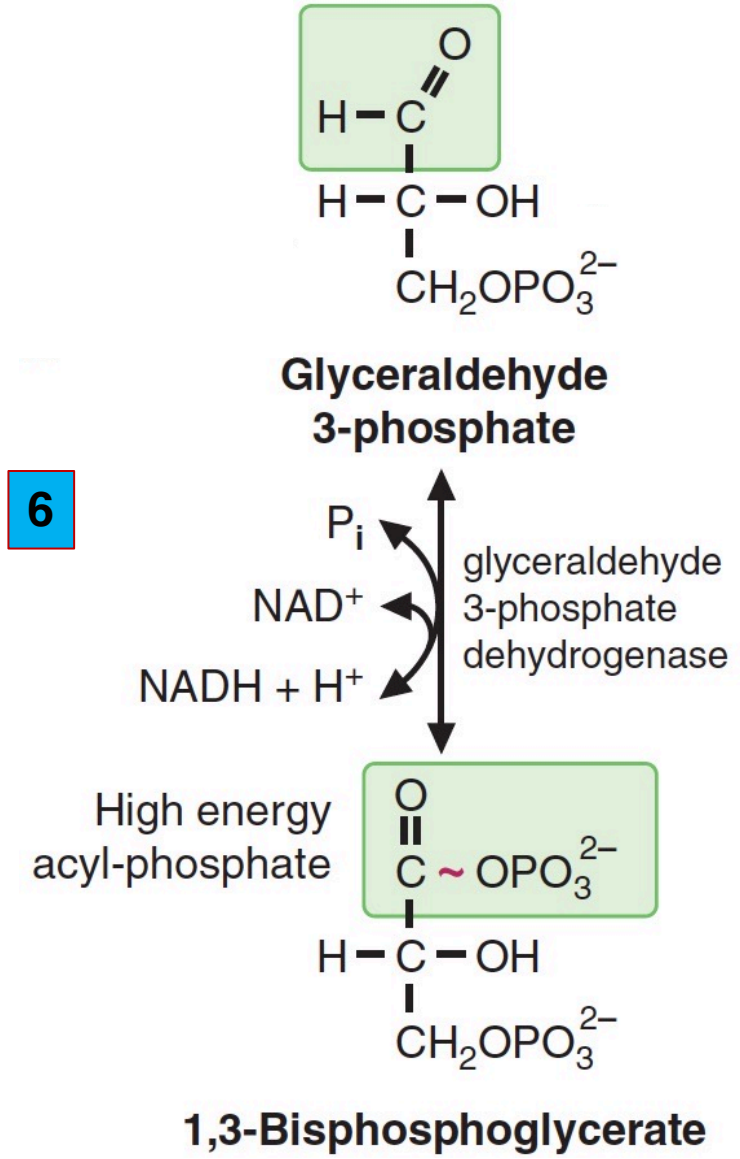
Reactions in Glycolysis

Step 6: Glyceraldehyde-3-phosphate is oxidized by NAD^+ and reacts with inorganic phosphate to form **1,3-bisphosphoglycerate** and NADH + H^+.

Enzyme: glyceraldehyde-3-phosphate dehydrogenase.

The aldehyde group of glyceraldehyde-3-phosphate is oxidized to a carboxylic acid, which forms a high-energy anhydride with inorganic phosphate.

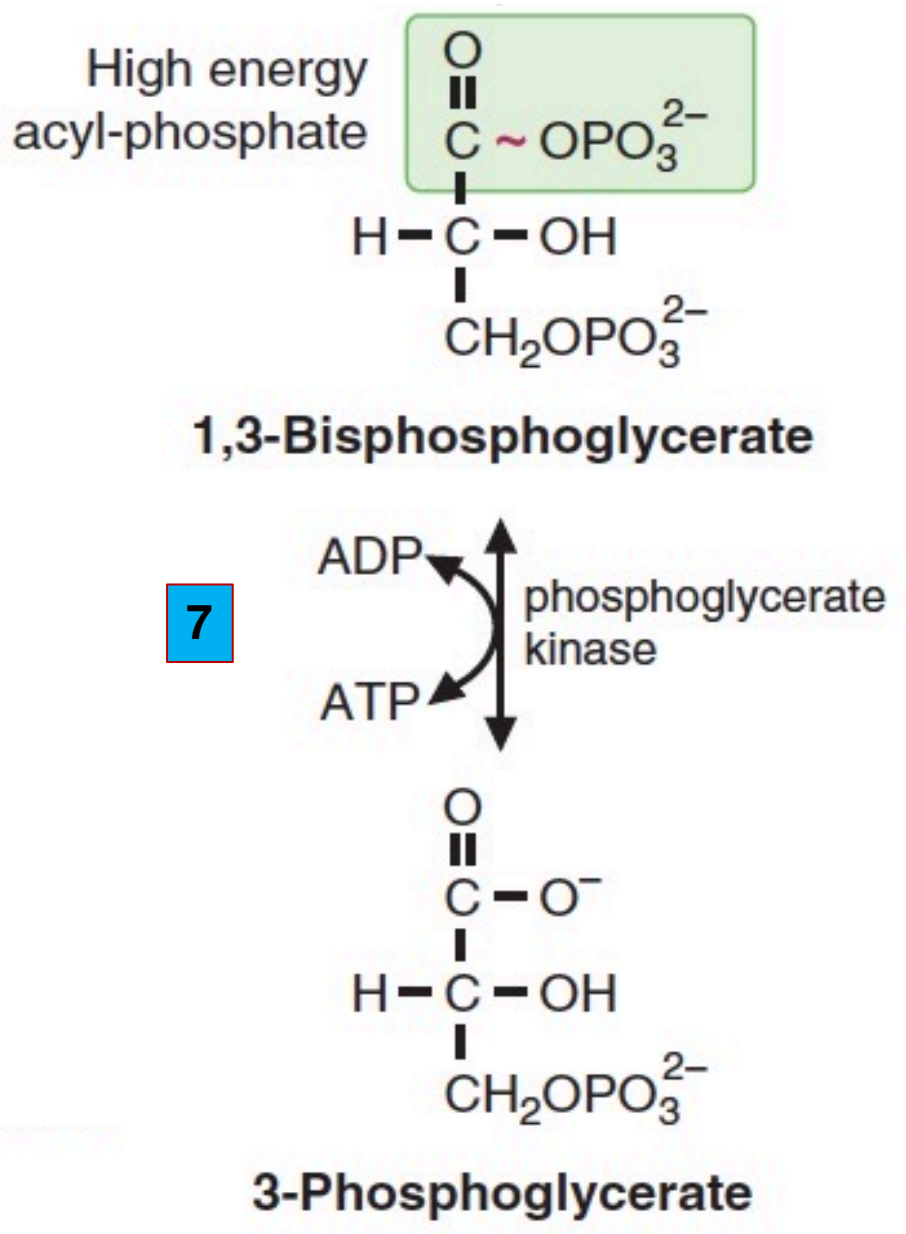
A cysteine residue at the active site is essential for this reaction to proceed.



Reactions in Glycolysis

Step 7: 1,3-Bisphosphoglycerate reacts with ADP to produce 3-phosphoglycerate and ATP.

Enzyme: phosphoglycerate kinase

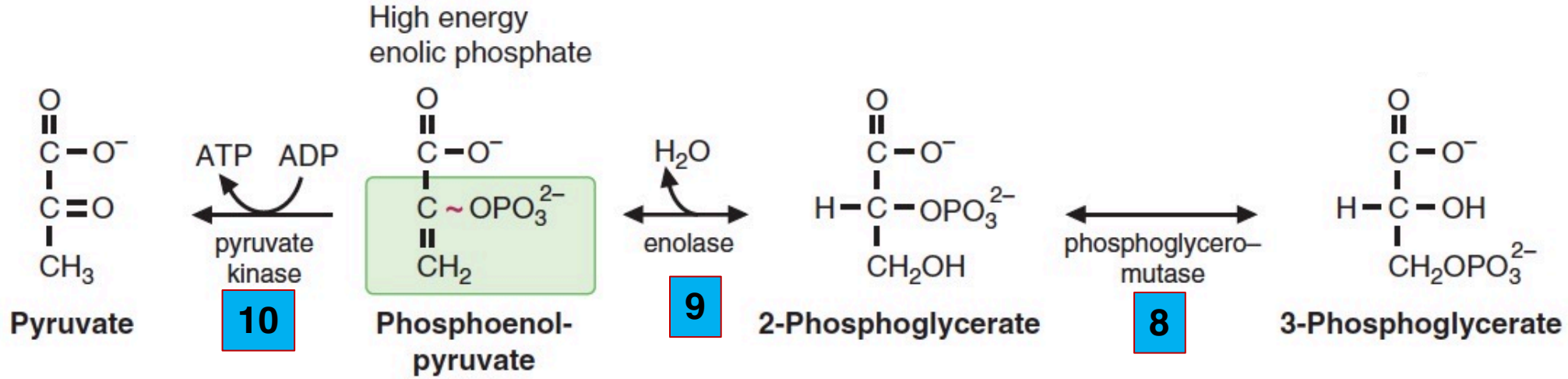


Reactions in Glycolysis

Step 8: 3-Phosphoglycerate is converted to 2-phosphoglycerate by transfer of the phosphate group from carbon 3 to carbon 2.
Enzyme: phosphoglyceromutase

Step 9: 2-Phosphoglycerate is dehydrated to phosphoenolpyruvate (PEP), which creates a high energy enol phosphate.
Enzyme: enolase

Step 10: Phosphoenolpyruvate reacts with ADP to form pyruvate and ATP in the last reaction ofglycolysis.
Enzyme: pyruvate kinase
Pyruvate kinase is more active in the fed state than in the fasting state

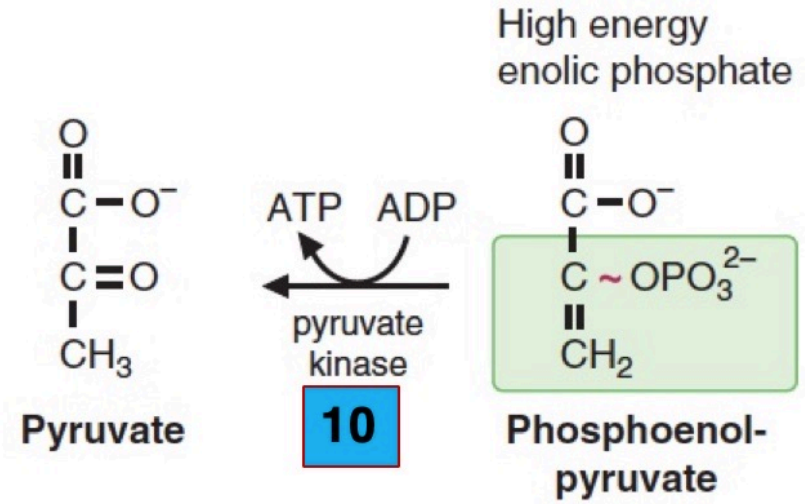


The summary of the reactions of the glycolytic pathway is that glucose + 2 NAD^+ + 2 P_i + 2 ADP yields 2 pyruvate + 2 NADH + 4 H^+ + 2 ATP + 2 H_2O.

Clinical correlates : pyruvate kinase deficiency

CLINICAL CORRELATES

Deficiency of pyruvate kinase causes decreased production of ATP from glycolysis. Red blood cells have insufficient ATP for their membrane pumps, and a **hemolytic anemia** results, although oxygen delivery to tissues is not necessarily affected. As phosphoenolpyruvate accumulates, it is converted to 2-phosphoglycerate, which leads to increased levels of 2,3-bisphosphoglycerate in the red blood cells. The elevated levels of 2,3-bisphosphoglycerate promote oxygen release from hemoglobin in the tissues to an extent that is greater than in the presence of normal 2,3-bisphosphoglycerate levels.



Regulation of Glycolysis: Hexokinase

1. Hexokinase is found in most **tissues**, and is geared to provide glucose-6-phosphate for ATP production even when blood glucose levels are low.

a. Hexokinase has a **low K_m** for glucose (about 0.1 mM). Therefore, it is working near its maximum rate (V_{max}), even at fasting blood glucose levels (about 5 mM).

b. Hexokinase is **inhibited by its product, glucose-6-phosphate**. Therefore, it is most active when glucose-6-phosphate is being rapidly utilized.

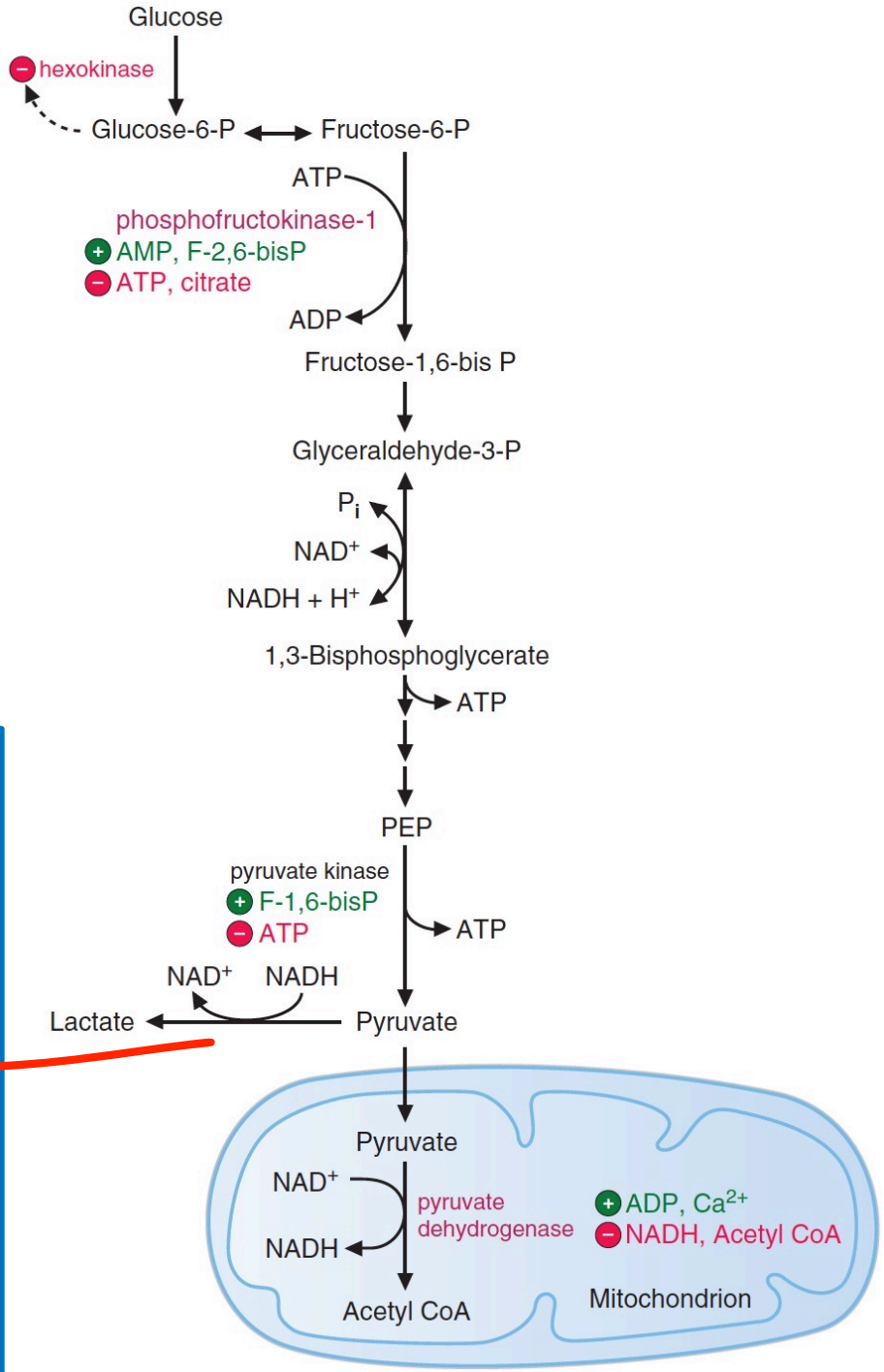
2. Glucokinase is found in the **liver and pancreas** and functions at a significant rate only after a meal.

a) Glucokinase has a high K_m for glucose (about 6 mM). Therefore, it is very active after a meal when glucose levels in the hepatic portal vein are high, and it is relatively inactive during fasting when glucose levels are low.

b) Glucokinase is **induced when insulin levels are high.**

c) Glucokinase is not inhibited by its product, glucose-6-phosphate, at physiologic concentrations.

d) Glucokinase is **regulated by a glucokinase regulatory protein**, which binds to glucokinase at low glucose concentrations and sequesters glucokinase in the nucleus. When glucose levels increase, the glucokinase is brought back to the cytoplasm and released from the regulatory protein.

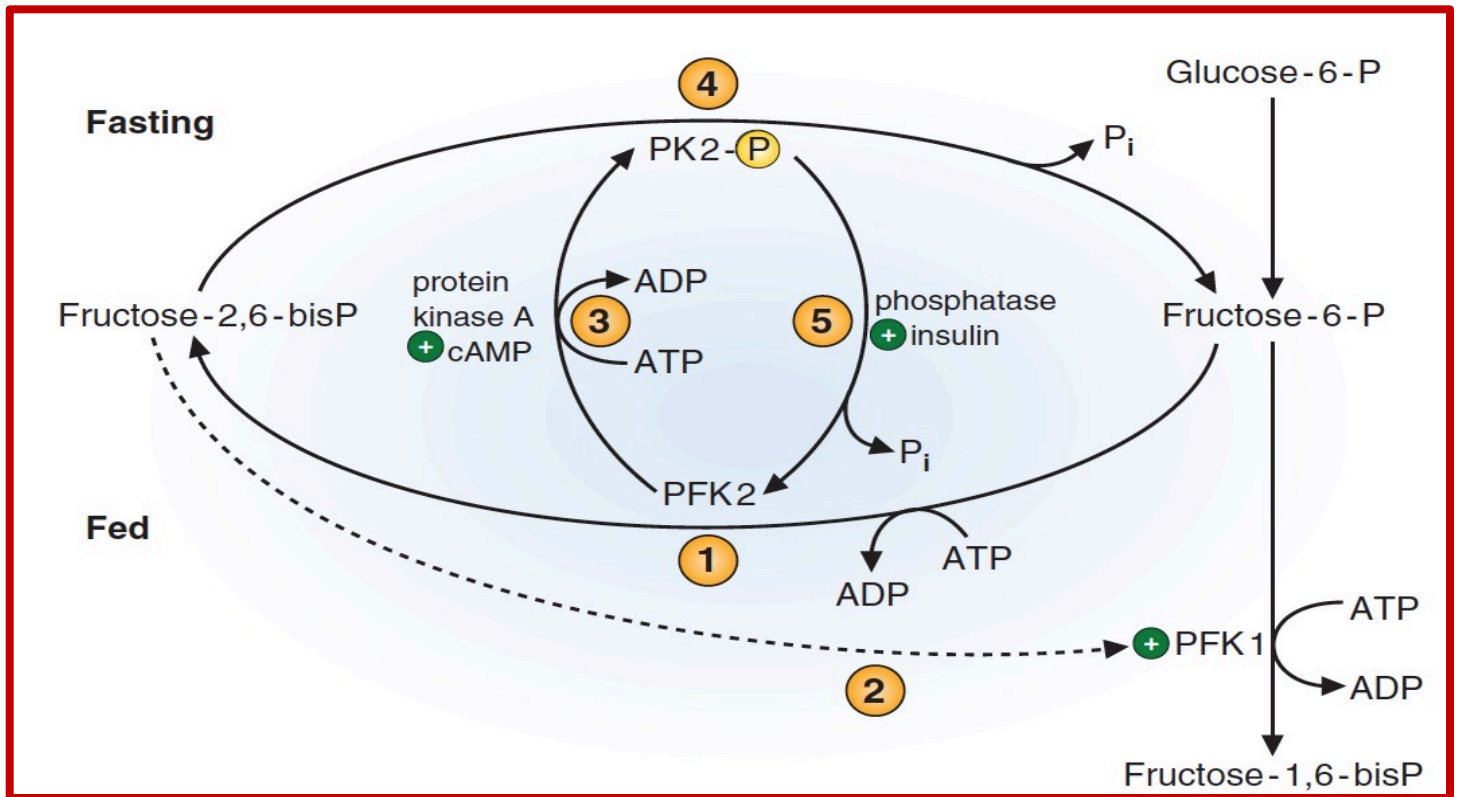


Regulation of Glycolysis: PFK1

3. PFK1 is regulated by several factors. It **functions at a rapid rate in the liver when blood glucose levels are high or in cells such as muscle when there is a need for ATP.**

a. **PFK1 is activated by fructose 2,6-bisphosphate (F-2,6-P),** an important regulatory mechanism in the **liver.**

1. **After a meal,** F-2,6-P is formed from F-6-P by phosphofructokinase 2 (PFK2).
2. F-2,6-P activates PFK1, and glycolysis is stimulated. The liver is using glycolysis to produce fatty acids for triacylglycerol synthesis.
3. In the **fasting state** (when glucagon is elevated), PFK2 is phosphorylated by protein kinase A, which is activated by cAMP.
4. Phosphorylated PFK2 converts F-2,6-P to fructose-6-phosphate. F-2,6-P levels fall, and PFK1 is less active.
5. **In the fed state**, **insulin causes phosphatases** (such as PP-1) to be stimulated. A phosphatase dephosphorylates PFK2, causing it to become more active in forming F-2,6-P from fructose-6-phosphate. F-2,6-P levels rise, and PFK1 is more active.



** Thus, **PFK2 acts as a kinase (in the fed state** when it is dephosphorylated) and as a phosphatase (in the fasting state when it is phosphorylated).

** The muscle isozyme of PFK2 is not regulated by phosphorylation, although the heart isozyme is, and in the heart the kinase activity of PFK2 is activated upon phosphorylation (the opposite of what occurs in the liver).

Regulation of Glycolysis: PFK 1 Cont…

b. PFK1 is activated by AMP, an important regulatory mechanism in muscle

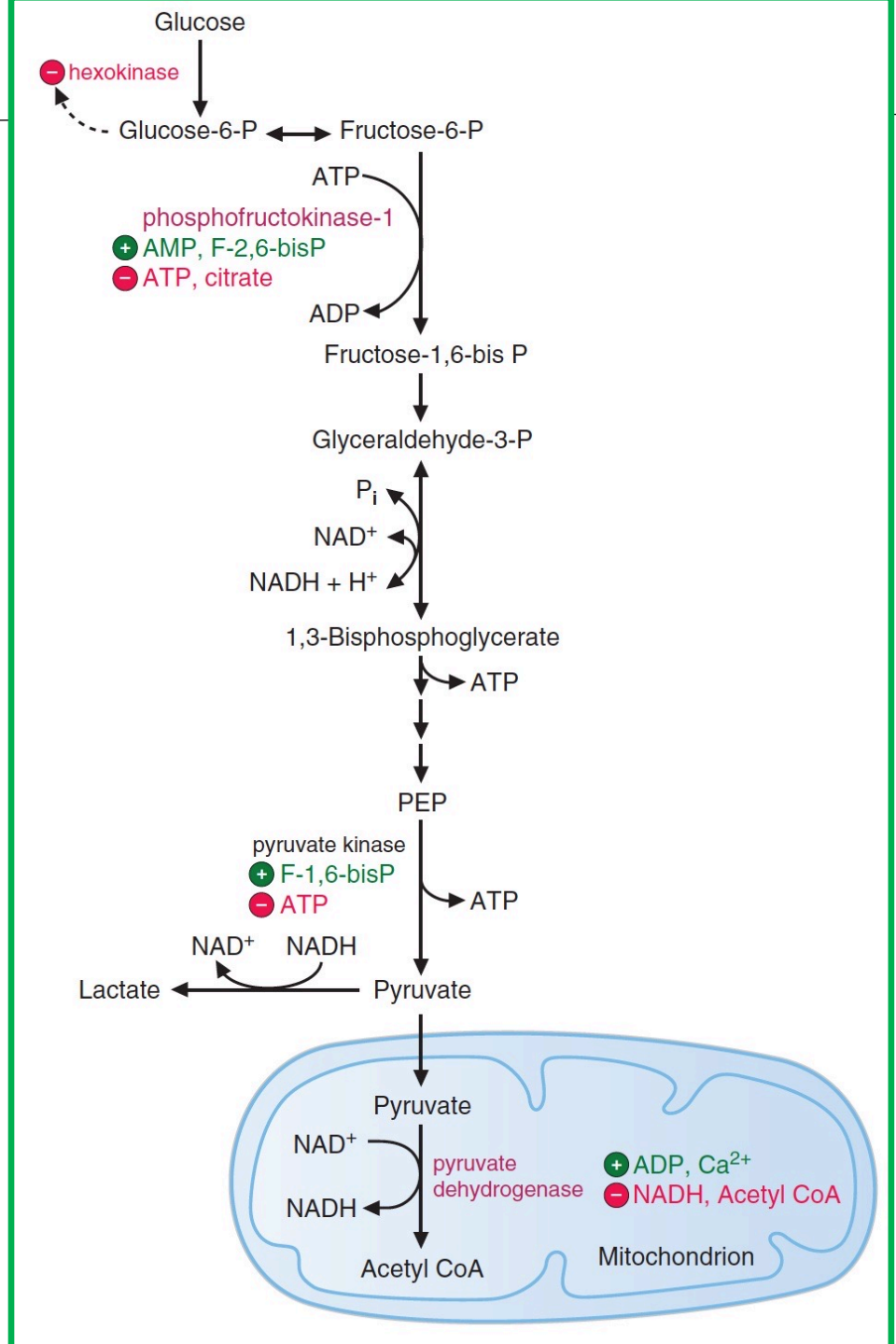
i. In muscle during exercise, AMP levels are high and ATP levels are low.

ii. Glycolysis is promoted by a more active PFK1, and ATP is generated.

c. PFK1 is inhibited by ATP and citrate, the important regulatory mechanisms in muscle.

i. When ATP is high, the cell does not need ATP, and glycolysis is inhibited.

ii. High levels of citrate indicate that adequate amounts of substrate are entering the TCA cycle, and that intramitochondrial levels of NADH and ATP are high. Therefore, glycolysis slows down.



Regulation of Glycolysis: pyruvate kinase

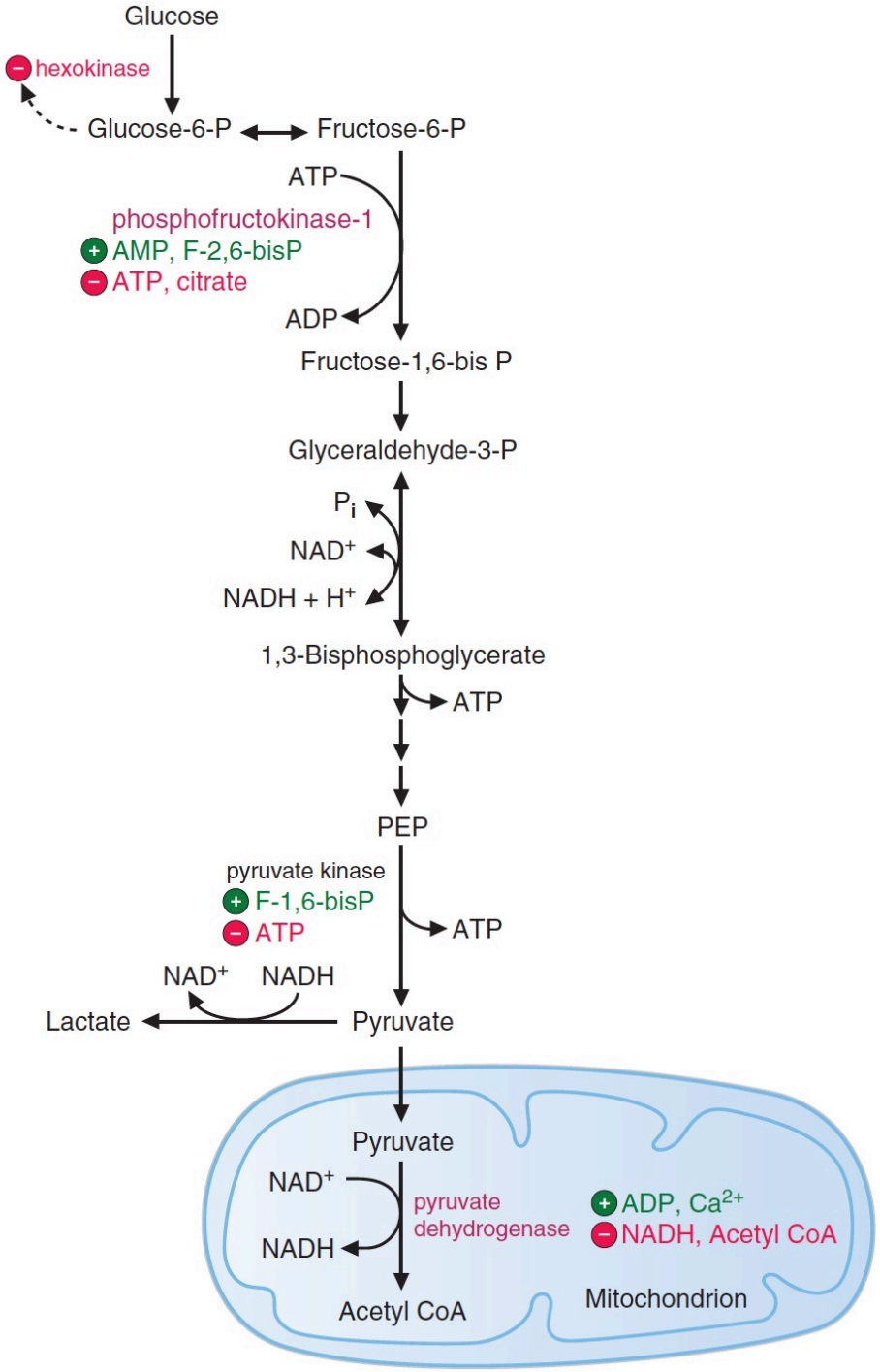
4. Pyruvate kinase

a. Pyruvate kinase **is activated by fructose 1,6-bisphosphate** and **inhibited by ATP** in the liver during fasting when glucagon levels are high.

i. Glucagon via cAMP activates protein kinase A, which phosphorylates and inactivates pyruvate kinase.
ii. The inhibition of pyruvate kinase promotes gluconeogenesis.
iii. The muscle isozyme of pyruvate kinase is not regulated by phosphorylation.

b. Pyruvate kinase **is activated in the fed state.**

i. Insulin stimulates phosphatases that dephosphorylate and activate pyruvate kinase.



Generation of ATP by glycolysis ➔ Anaerobic conditions

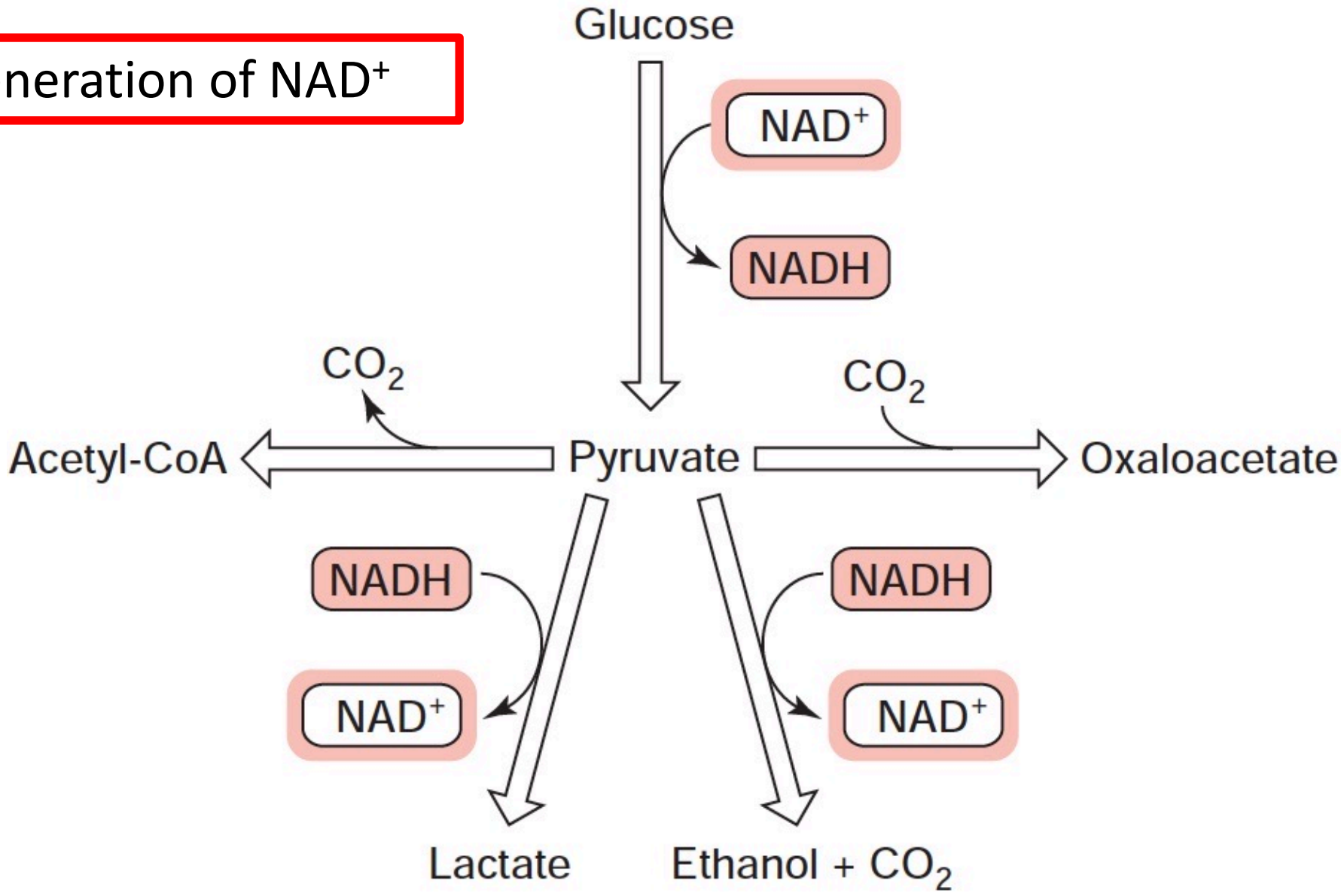
1. Production of ATP and NADH in the glycolytic pathway: Overall, when 1 mole of glucose is converted to **2 moles of pyruvate**, 2 moles of ATP are used in the process, and 4 moles of ATP are produced, for a **net yield of 2 moles of ATP**. In addition, **2 moles of cytosolic NADH** are generated.

2. Energy generated by conversion of glucose to lactate

If the NADH generated by glycolysis is used to reduce pyruvate to lactate, the net yield is 2 moles of ATP per mole of glucose converted to lactate.

Fate of pyruvate under anaerobic conditions: fermentations

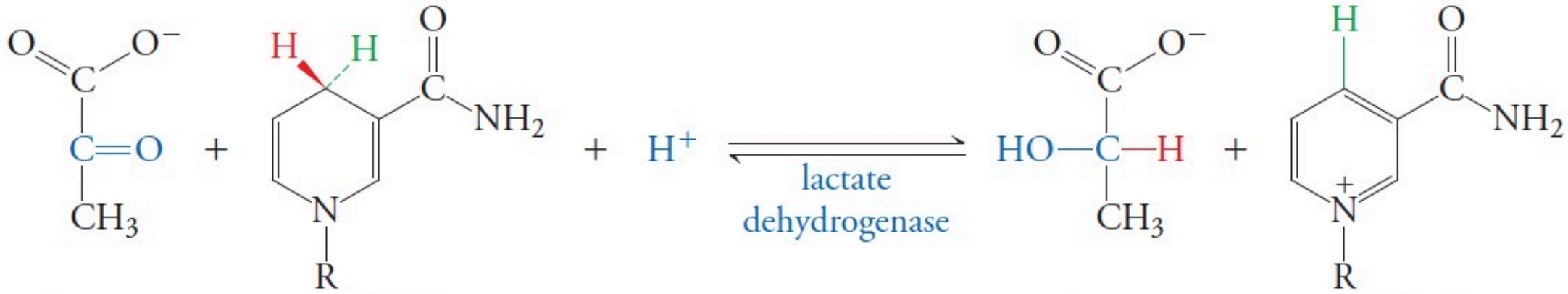
Overall Aim: ➔ regeneration of NAD^+



The fate of pyruvate

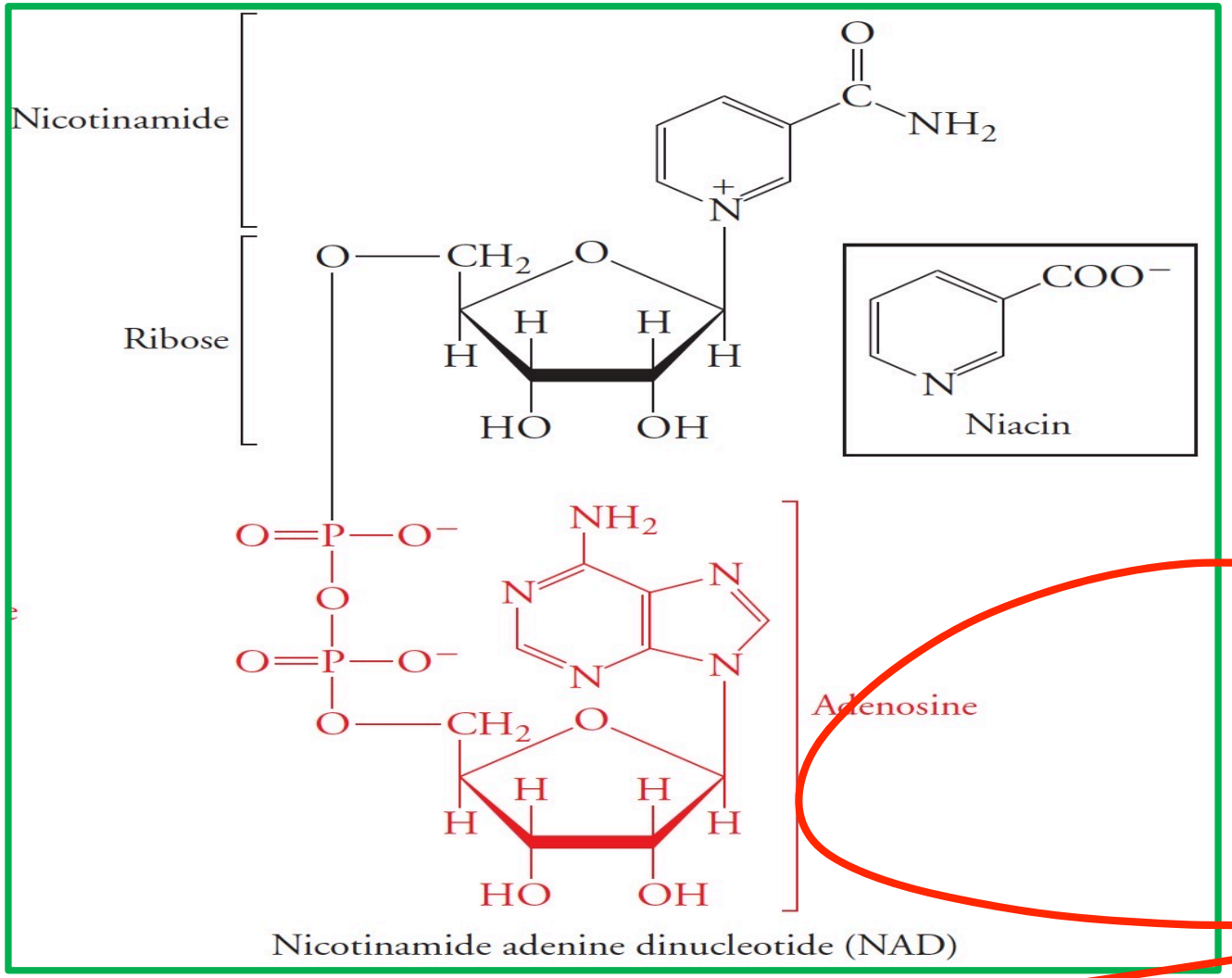
1. Conversion to lactate

a. Pyruvate can be reduced in the cytosol by NADH, forming lactate, and regenerating NAD^+. The enzyme is lactate dehydrogenase (LDH).



NADH, which is produced by glycolysis, must be reconverted to NAD^+ so that carbons of glucose can continue to flow through glycolysis. This is particularly important under **anaerobic conditions.**

Structures of NAD



Nicotinamide adenine dinucleotide (NAD) is a derivative of the vitamin niacin (also called nicotinic acid or **vitamin B3**; see inset) and undergoes oxidation and reduction. The related compound nicotinamide adenine dinucleotide phosphate (NADP) contains a phosphoryl group at the adenosine C2' position.

The fate of pyruvate

1. Conversion to lactate…continued

II. LDH converts pyruvate to lactate. LDH consists of four subunits that can be either of the muscle (M) or the heart (H) type.

a) Five isozymes occur which can be separated by electrophoresis.

- MMMM
- MMMH
- MMHH
- MHHH
- HHHH

b) Different tissues have different mixtures of these isozymes.

III. Lactate is released by tissues (e.g., red blood cells or exercising muscle) and is used by the **liver for gluconeogenesis** or **by tissues such as the heart and kidney where it is converted to pyruvate and oxidized for energy.**

Clinical correlates (lactic acidosis)

CLINICAL CORRELATES

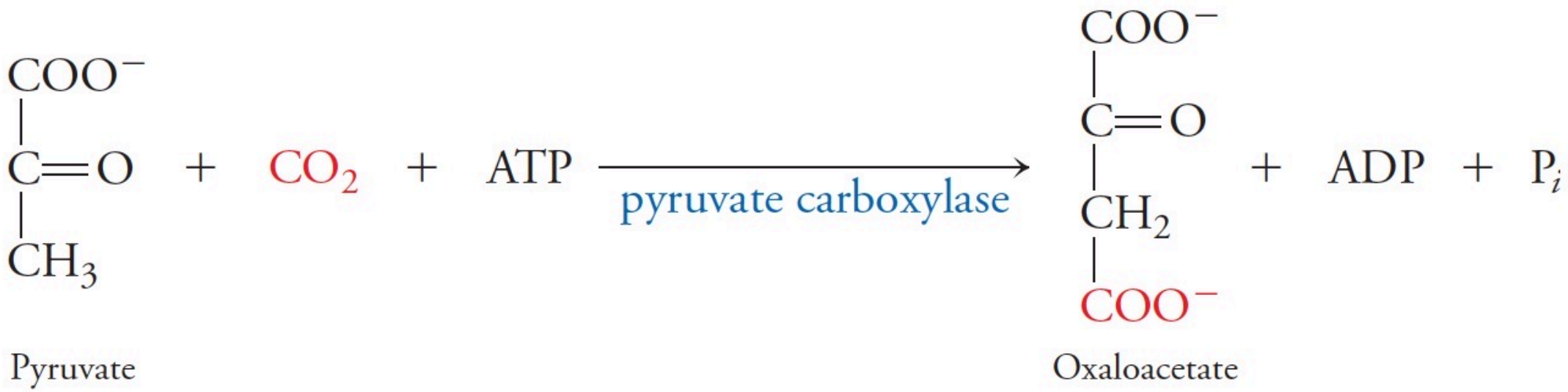
An increase of lactate levels in the blood causes an **acidosis (lactic acidosis)**. This condition can result from **hypoxia** or **alcohol ingestion**. Lack of oxygen slows down the electron transport chain, resulting in increased NADH levels. High NADH levels cause more than normal amounts of pyruvate to be converted to lactate. High NADH levels from alcohol metabolism also cause increased conversion of pyruvate to lactate. **Thiamine deficiency**, which is common in alcoholics, decreases pyruvate dehydrogenase activity, causing pyruvate to accumulate and form lactate. Thiamine deficiency also slows down the TCA cycle at the α-ketoglutarate dehydrogenase step. This and other conditions that slow down the TCA cycle can also produce a **lactic acidosis**.

The fate of pyruvate

2. Conversion to oxaloacetate

- Pyruvate can be converted to oxaloacetate by pyruvate carboxylase, an enzyme found in tissues such as the liver, brain and muscle

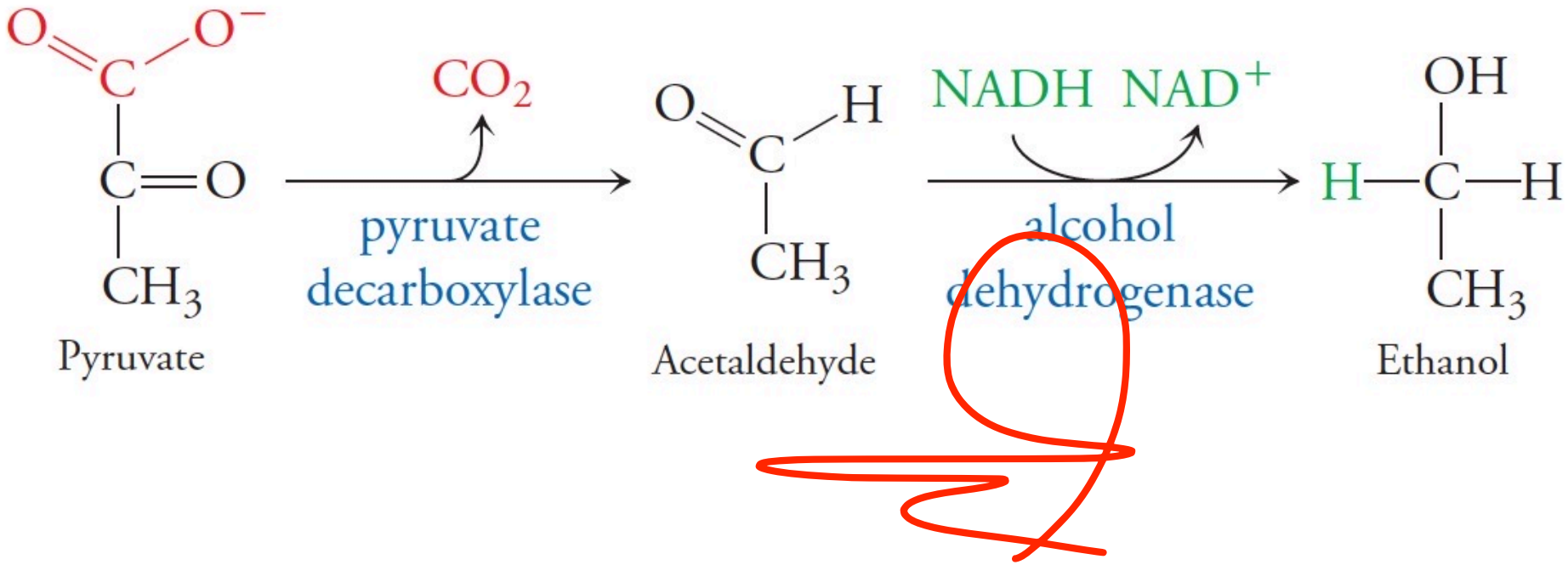
- This reaction serves to **replenish** the intermediates of the TCA cycle.



The fate of pyruvate

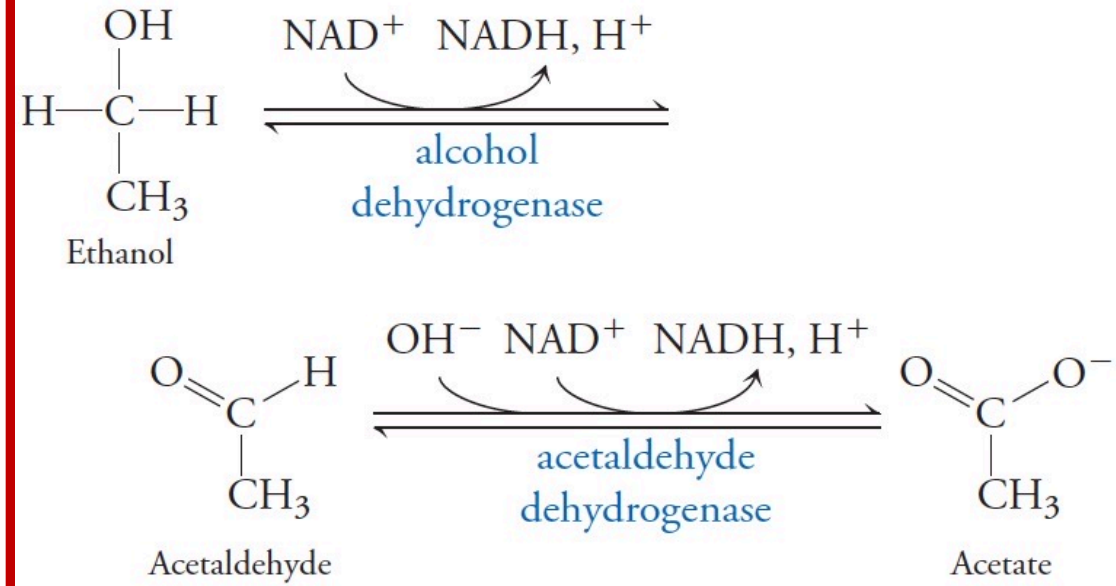
3. Conversion to ethanol

** This occurs in yeast



Clinical correlates: Alcohol induced “hangover” and liver cirrhosis

Unlike yeast, mammals do not produce ethanol, although it is naturally present in many foods and is produced in small amounts by intestinal microorganisms.
The liver is equipped to metabolize ethanol, a small, weakly polar substance that is readily absorbed from the gastrointestinal tract and transported by the bloodstream.
First, alcohol dehydrogenase converts ethanol to acetaldehyde. This is the reverse of the reaction yeast use to produce ethanol. A second reaction converts acetaldehyde to acetate:



The unpleasant symptoms of a **hangover** in part reflect the chemistry of producing **acetaldehyde and acetate**. As shown above, their production in the liver consumes NAD^+, thereby **lowering the cell’s NAD^+:NADH ratio.** Without sufficient NAD^+, the liver’s ability to produce ATP by glycolysis is diminished (since NAD^+ is required for the glyceraldehyde-3-phosphate dehydrogenase reaction). Acetaldehyde itself can react with liver proteins, inactivating them. Acetate (acetic acid) production lowers blood pH. **Long-term, excessive alcohol consumption** exacerbates the toxic effects of ethanol and its metabolites. For example, a shortage of liver NAD^+ slows fatty acid breakdown leading to fat accumulation in the liver. Over time, cell death causes permanent loss of function in the central nervous system. The death of liver cells and their replacement by fibrous scar tissue causes liver **cirrhosis.**

Generation of ATP by glycolysis

1. Production of ATP and NADH in the glycolytic pathway

Overall, when 1 mole of glucose is converted to 2 moles of pyruvate, 2 moles of ATP are used in the process, and 4 moles of ATP are produced, for a net yield of 2 moles of ATP. In addition, 2 moles of cytosolic NADH are generated.

2. Energy generated by conversion of glucose to lactate

If the NADH generated by glycolysis is used to reduce pyruvate to lactate, the net yield is 2 moles of ATP per mole of glucose converted to lactate.

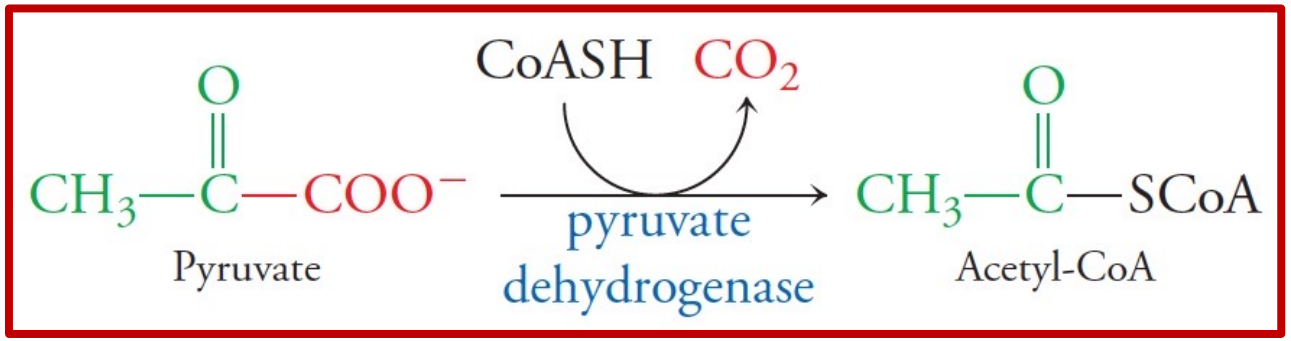
..

Fate of pyruvate under aerobic conditions➔ TCA cycle & Oxidative phosphorylation

The fate of pyruvate

2. Conversion to acetyl-CoA

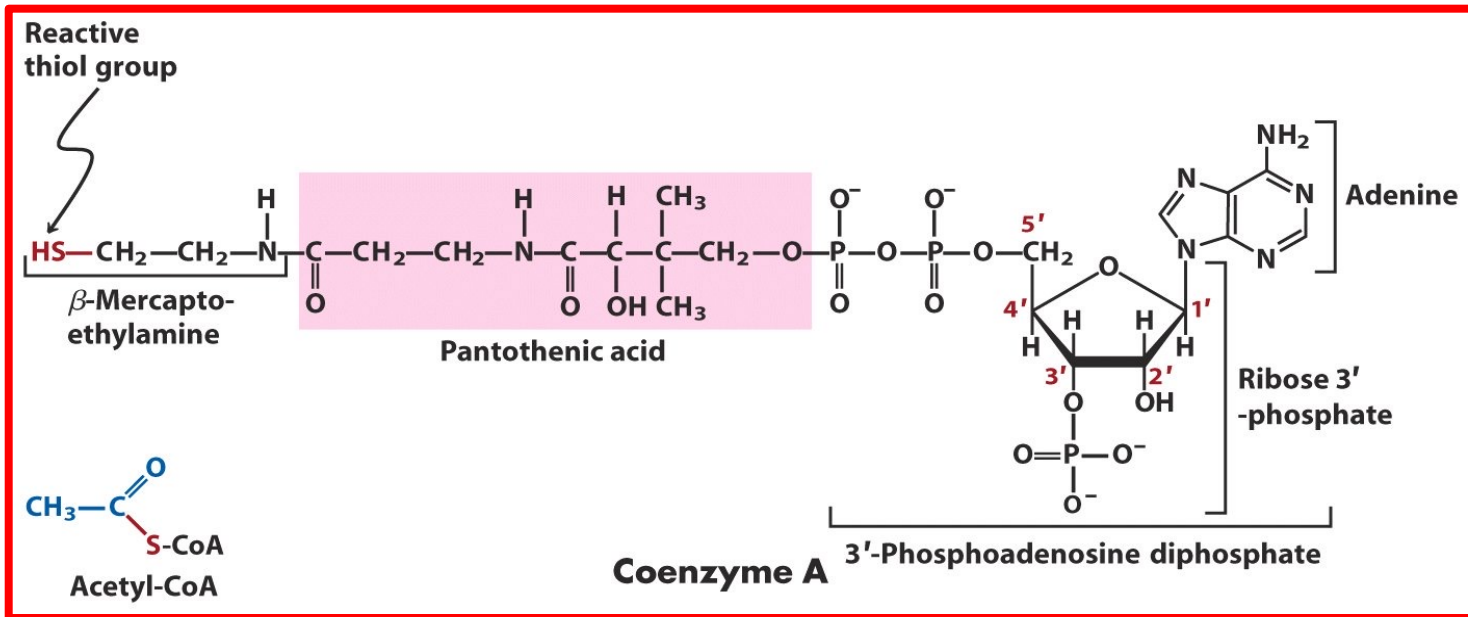
Pyruvate can enter mitochondria and be converted by **pyruvate dehydrogenase** to acetyl-CoA, which can enter the TCA cycle.



Coenzyme A (CoA) contains a residue of **pantothenic acid** (pantothenate), also known as **vitamin B5**. The sulfhydryl group is the site of attachment of other groups.

TABLE 17.1 Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide



Generation of ATP by glycolysis =➔ Aerobic conditions

3. Energy generated by conversion of glucose to CO_2 and H_2O

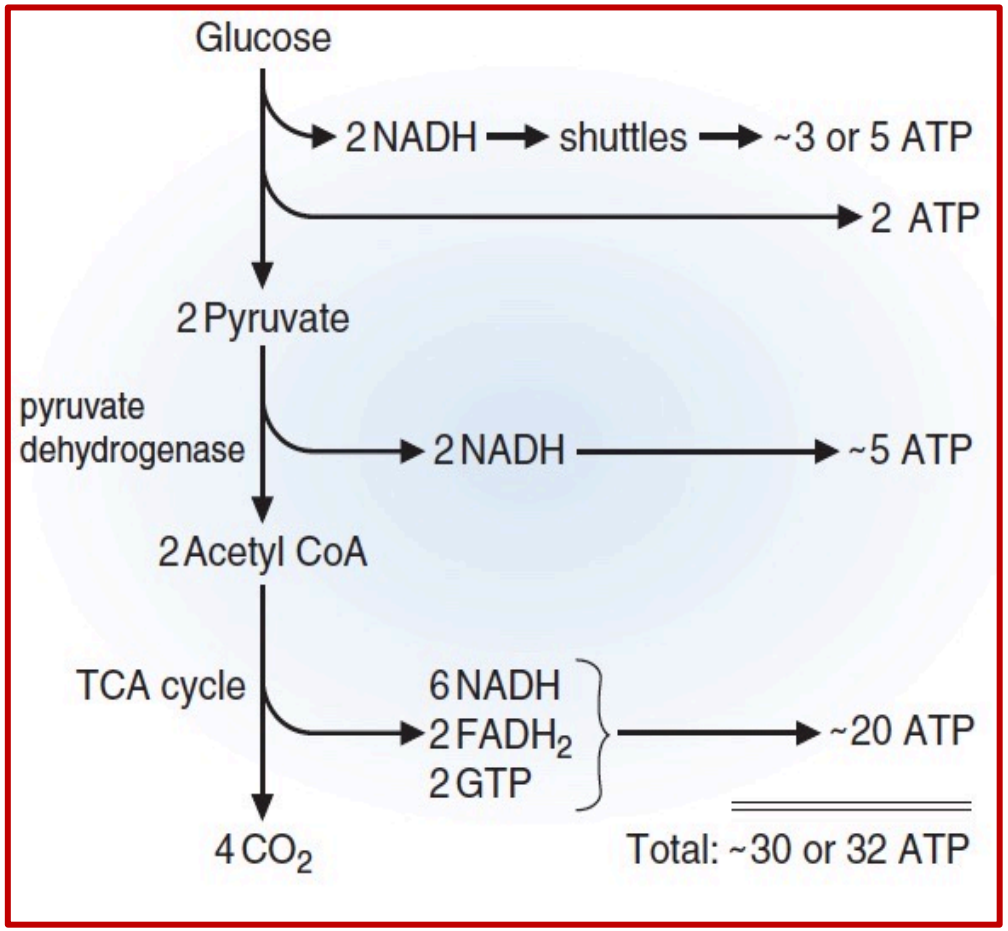
a. When glucose is oxidized completely to CO_2 and H_2O, approximately 30 or 32 moles of ATP are generated.

i. **Two moles of ATP** and **2 moles of NADH** are generated from the conversion of 1 mole of glucose to 2 moles of pyruvate.

ii. The **2 moles of pyruvate** enter the mitochondria and are converted to **2 moles of acetyl-CoA**, producing **6 moles of NADH** which generate approximately **15 moles of ATP** by oxidative phosphorylation.

iii. The **2 moles of acetyl-CoA** are oxidized in the TCA cycle, generating approximately **20 moles of ATP.**

iv. NADH, produced in the cytosol by glycolysis, cannot directly cross the mitochondrial membrane. Therefore, the electrons are passed to the mitochondrial electron transport chain by two shuttle systems: Glycerol phosphate shuttle & Malate aspartate shuttle



4. Maximal ATP production

a. Overall, when 1 mole of glucose is oxidized to CO_2 and H_2O, approximately **30 moles of ATP are produced if the glycerol phosphate shuttle** is used, or 32 moles if the malate aspartate shuttle is used.

Questions & Answers