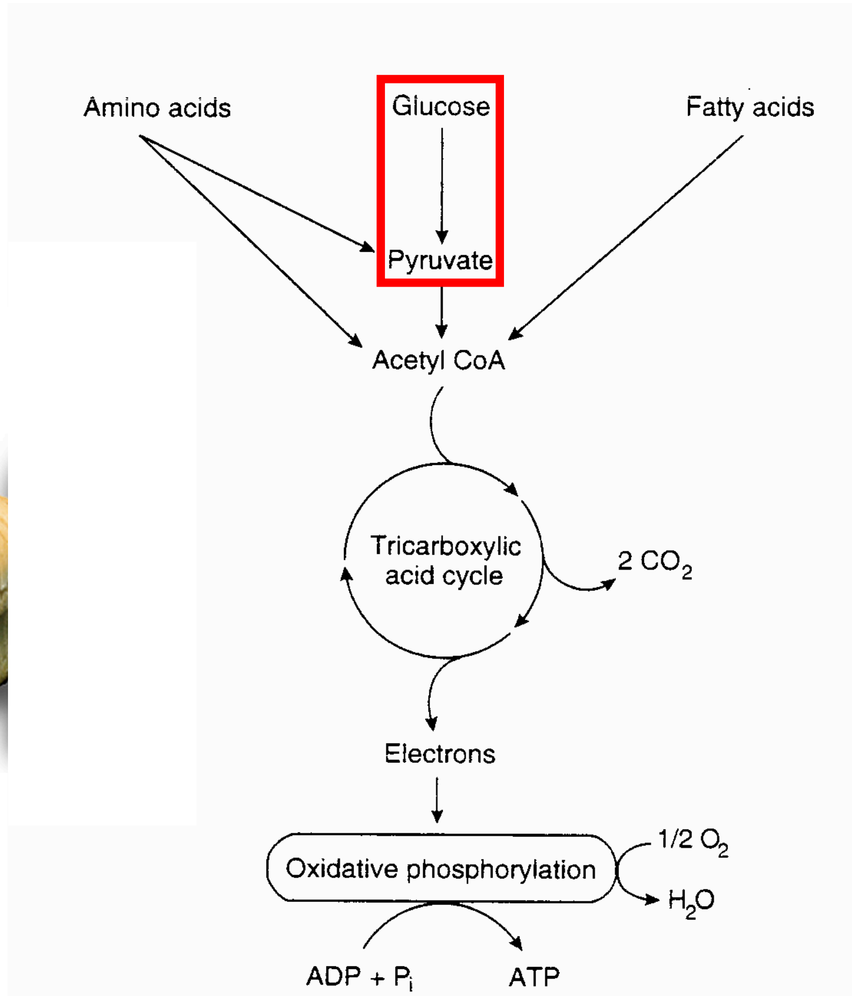
GLYCOLYSIS

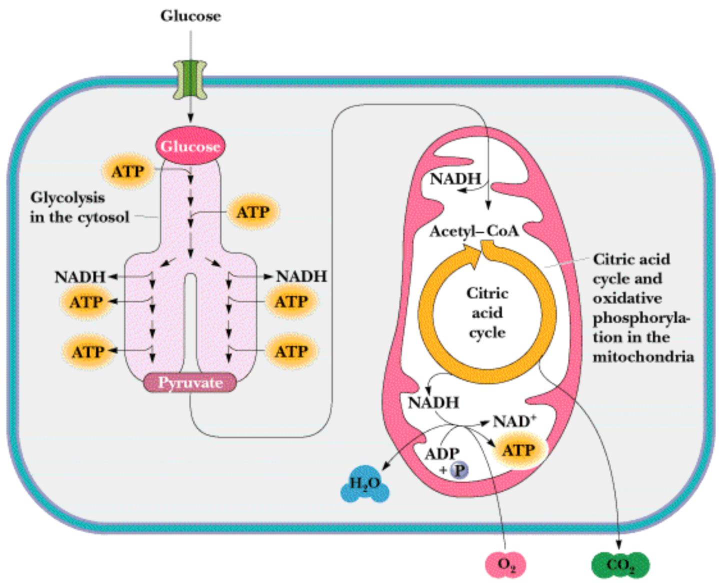
Generation of ATP from Metabolic Fuels



- **Catabolic process – degradative pathway**
- Energy stored in sugars (carbohydrates) released to perform biological work
- Transforms GLUCOSE to PYRUVATE under ANAEROBIC conditions
- Glucose enters the cell via a specific transporter protein
- **Uses:**
  - Glucose
  - ATP
  - ADP + Pi
  - NAD^+ (necessary co-factor)

- **Produces:**
  - Pyruvate
  - ATP
  - NADH – can be further oxidized under *aerobic* conditions to make ATP

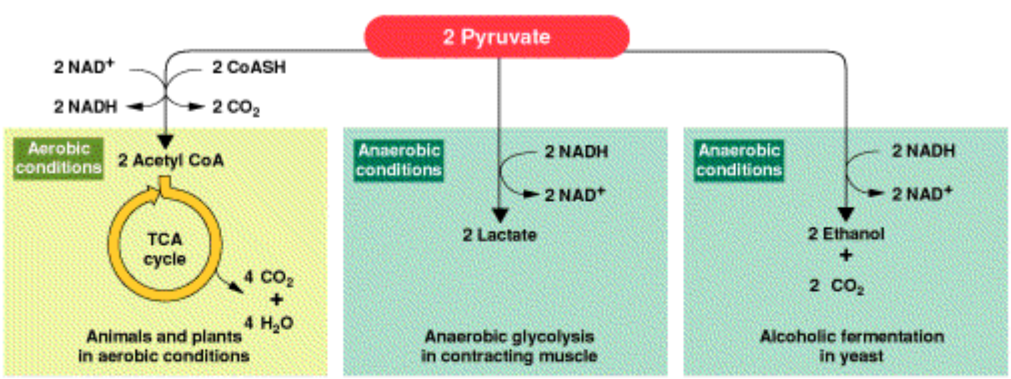
Reactions of **glycolysis** occurs in the **CYTOSOL**



- **THREE FATES OF PYRUVATE**
  - **Aerobic conditions**
    - conversion to acetyl CoA (pyruvate dehydrogenase) for use in TCA cycle and oxidative phosphorylation for ATP production
  - **Anaerobic conditions**
    - Lactate (animal muscles)
    - Ethanol (yeast)

TABLE 13-1 | Standard Free Energy Changes for Glucose Catabolism

Catabolic Process	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
$C_6H_{12}O_6 \rightarrow 2\,C_3H_5O_3^- + 2\,H^+$ (glucose) (lactate)	−196
$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,H_2O + 6\,H_2O$ (glucose)	−2850



- **ANABOLIC PROCESS: GLUCONEOGENESIS**
  - Synthesize glucose FROM pyruvate or lactate
  - Increases free glucose concentration

OVERALL REACTION FOR GLYCOLYSIS:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{NAD}^+ + 2\ \text{P}_i \longrightarrow$$
$$2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O}$$

- 10 Step Process – some steps tightly regulated
- Each glucose (6 carbons) split into TWO pyruvates (3 carbons each)
- Two molecules of ATP are produced
- Two molecules of NAD+ are reduced to NADH

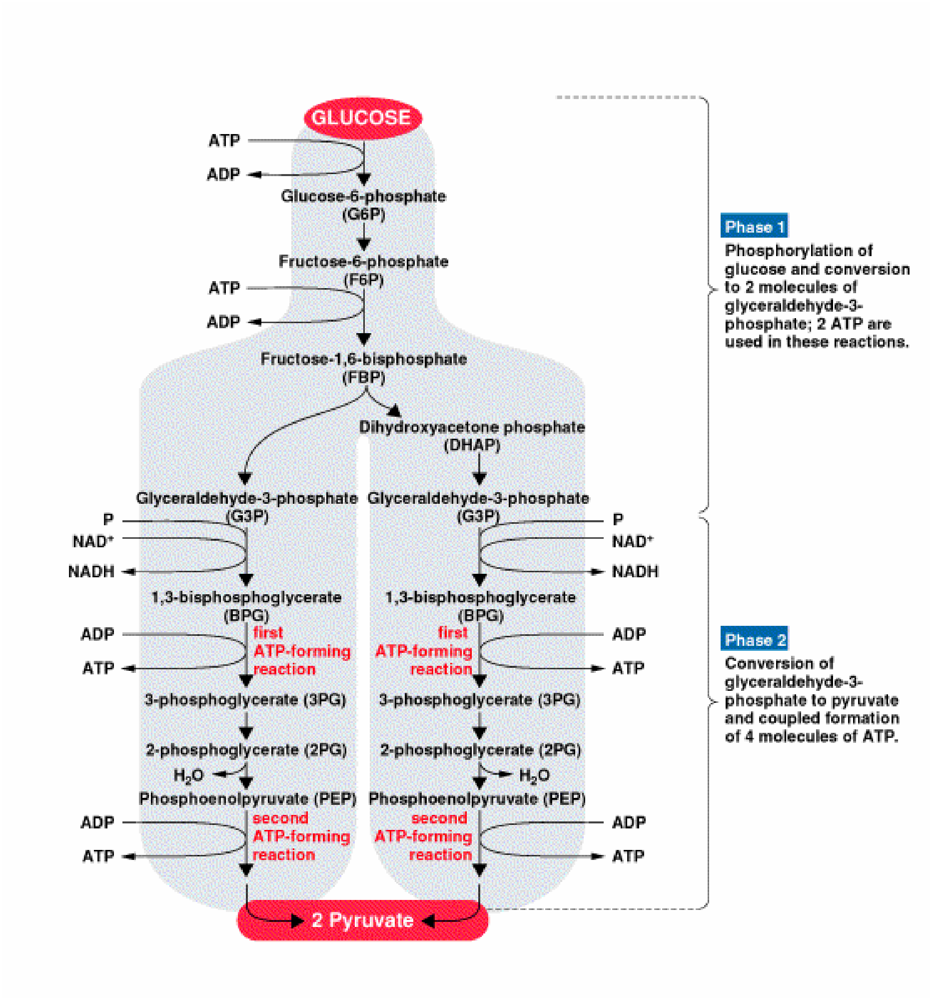
- **TWO PHASES:**
    - **INVESTMENT PHASE**
        - First 5 reactions
        - Glucose is activated by phosphorylation
            - "Priming reactions" – need to invest energy to get more out
        - Uses 2 ATP's per glucose
        - Glucose is converted to TWO molecules of glyceraldehyde 3-phosphate (G3P)

    - **DIVIDEND PHASE**
        - Second set of 5 reactions
        - Each glyceraldehyde 3-phosphate (G3P) → pyruvate
        - Get FOUR ATP's out
        - Net gain of 2 ATP's

- **Modest return of energy! Will see big return once pyruvates enter TCA cycle and oxidative phosphorylation.**

HANDOUT:

- **Not necessary to memorize structures except glucose and pyruvate**
- **Know types of enzymes and recognize names of intermediates and enzymes**
- **Know regulatory steps**
- **Be able to count ATP's and follow what is made or used when and where.**



INVESTMENT

DIVIDEND

NET:
2 ATP per GLUCOSE
2 NADH per GLUCOSE

Table 15.1
The reactions of glycolysis with common enzyme names and reaction type

Reaction Number	Reaction	Enzyme[a]	Reaction Type[b]
1	Glucose + ATP → glucose-6-phosphate + ADP	Hexokinase	2
2	Glucose-6-phosphate ⇌ fructose-6-phosphate	Phosphoglucoisomerase	5
3	Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP	Phosphofructokinase	2
4	Fructose-1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	Aldolase	4
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde-3-phosphate	Triose phosphate isomerase	5
6	Glyceraldehyde-3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + $NADH^+$	Glyceraldehyde-3-phosphate dehydrogenase	1
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	2
8	3-Phosphoglycerate ⇌ 2-phosphoglycerate	Phosphoglycerate mutase	5
9	2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	Enolase	4
10	Phosphoenolpyruvate + ADP → pyruvate + ATP	Pyruvate kinase	2

[a] Enzymes are listed by common names.
[b] Reaction type: (1) oxidation–reduction, (2) phosphoryl group transfer, (3) hydrolysis, (4) nonhydrolytic cleavage (addition or elimination), (5) isomerization–rearrangement, and (6) bond formation coupled to ATP cleavage (see Section 14.2).

Table 15-1 Concepts in Biochemistry, 3/e

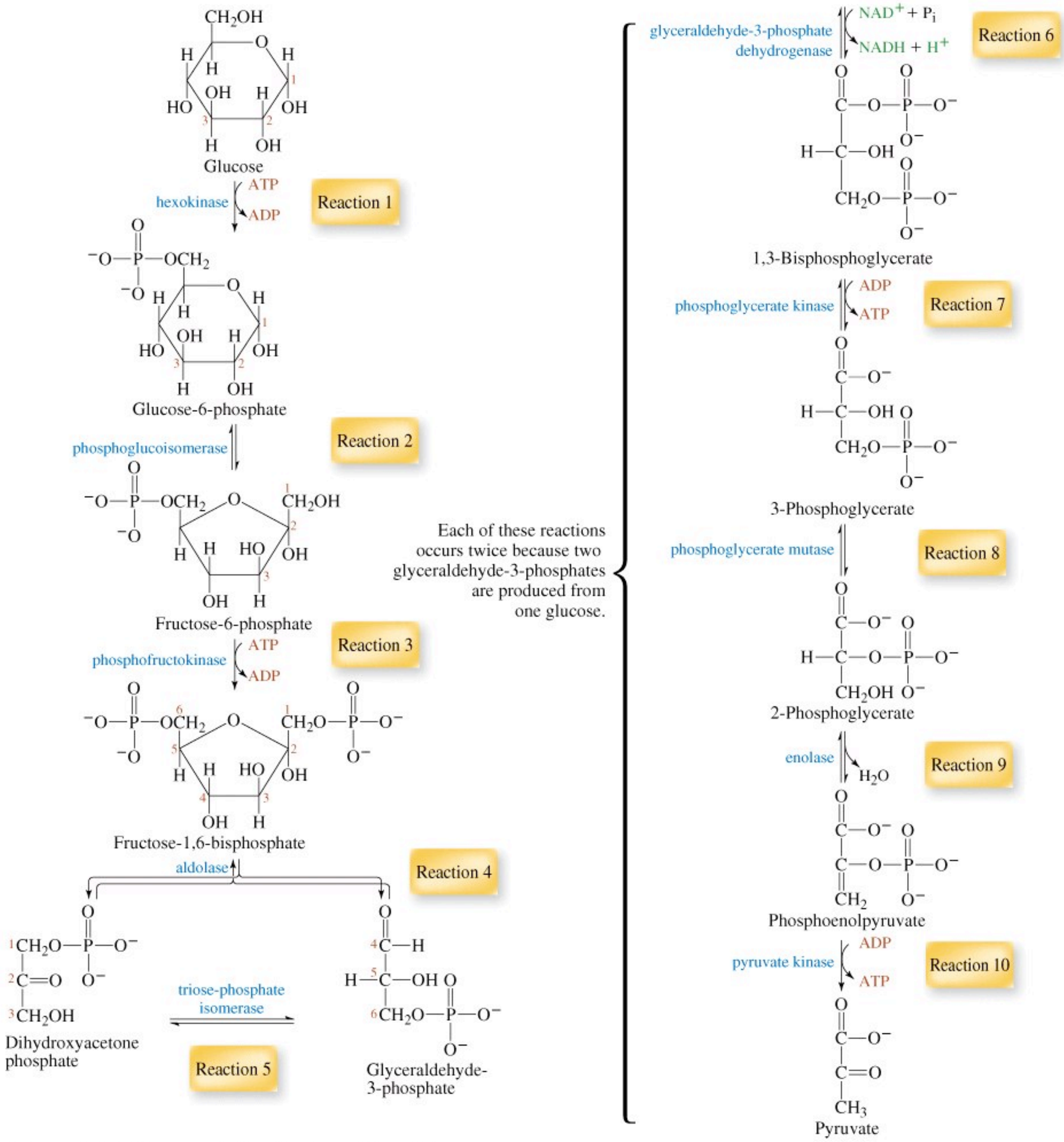
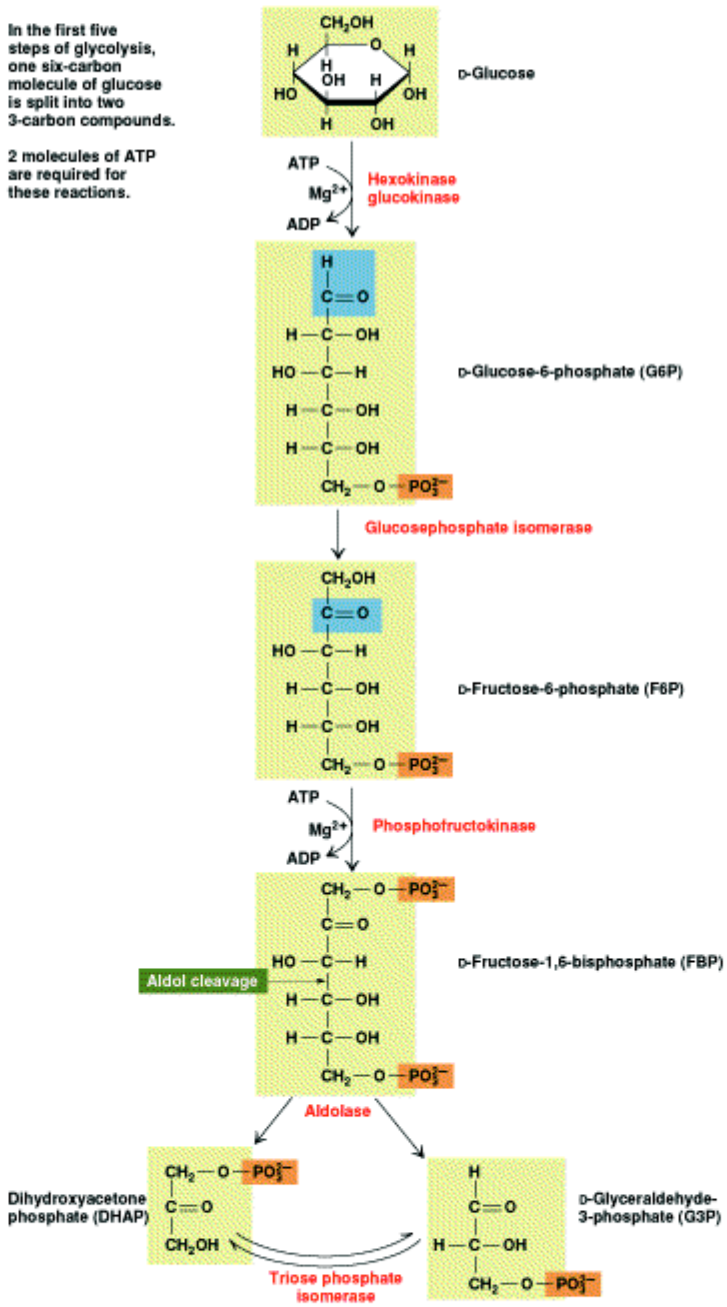
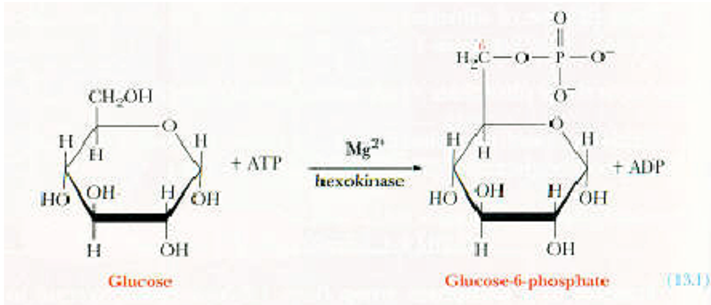


Figure 15-2 Concepts in Biochemistry, 3/e

REACTIONS OF GLYCOLYSIS IN DETAIL
Investment Phase

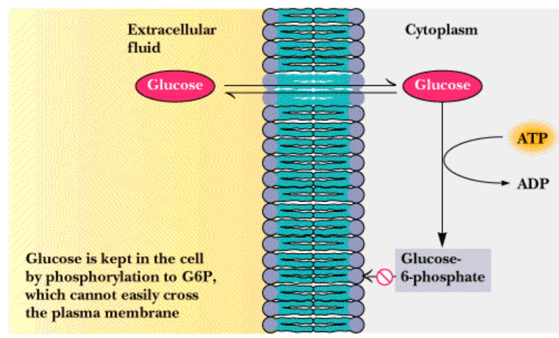


STEP 1: Glucose to glucose-6-phosphate

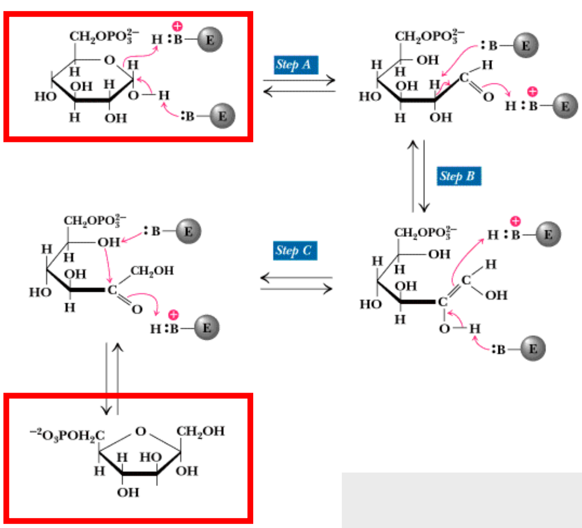


- Phosphorylation of glucose by **HEXOKINASE**
  - **KINASE** – Enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor substrate
  - Type of **TRANSFERASE** enzyme
  - Regulated but not the committed step
    - Glucose-6-phosphate can form glycogen or other pathways
- **ATP COUNT**
  - -1 (one ATP used)
  - 1st Investment of Energy

Phosphorylation keeps glucose in the cell



STEP 2: Glucose-6-phosphate to fructose-6-phosphate



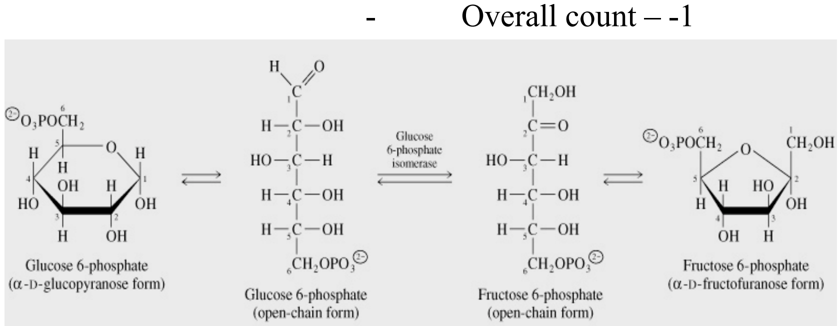
ENZYME: Phosphoglucose Isomerase

Type of **ISOMERASE** – Rearrangement of functional groups to form the isomer; ΔG – near zero; concentration of reactants and products affect direction.

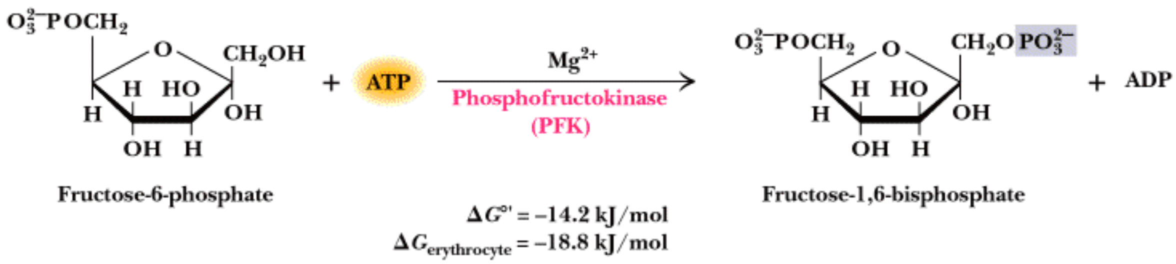
- Convert Glucose-6-phosphate to fructose-6-phosphate

- Not a regulated or committed step

- ATP COUNT:
- This step – 0
- Overall count – -1



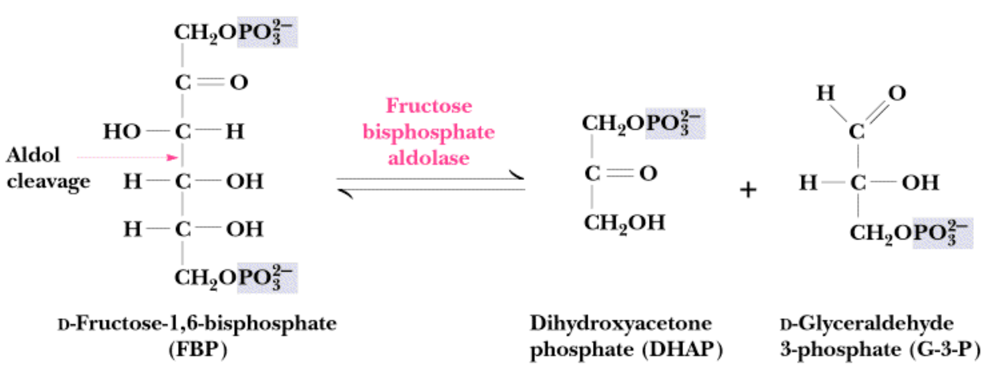
STEP 3: Fructose-6-phosphate to fructose-1,6-bisphosphate



- **ENZYME: Phosphofructokinase**
  - **KINASE** – same as first step; **TRANSFERASE** reaction
  - 2nd Investment of energy – one more ATP used
- **ATP COUNT:**
  - This step – -1
  - Overall count – -2
- **KEY CONTROL STEP – IRREVERSIBLE!!**
  - Committed step
  - Note HIGHLY negative ΔG°' – means *not* reversible

STEP 4:

Fructose-1,6-bisphosphate → glyceraldehyde-3-phosphate & dihydroxyacetone phosphate

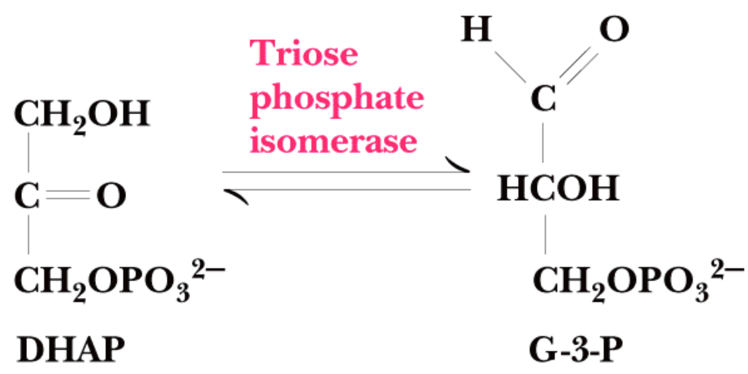


- **ENZYME: Aldolase**
  - **Non-hydrolytic Cleavage** reaction (type of **lyase**)
  - Cleaves glucose molecule into 2 molecules
  - $\Delta G^{0\prime}$ – +22.8 kJ/mol; *in vivo* ΔG is less than zero – products are quickly consumed. Rapid consumption of products pulls reaction forward.

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2

- **Not a regulatory step**

STEP 5: Dihydroxyacetone phosphate to glyceraldehyde-3-phosphate

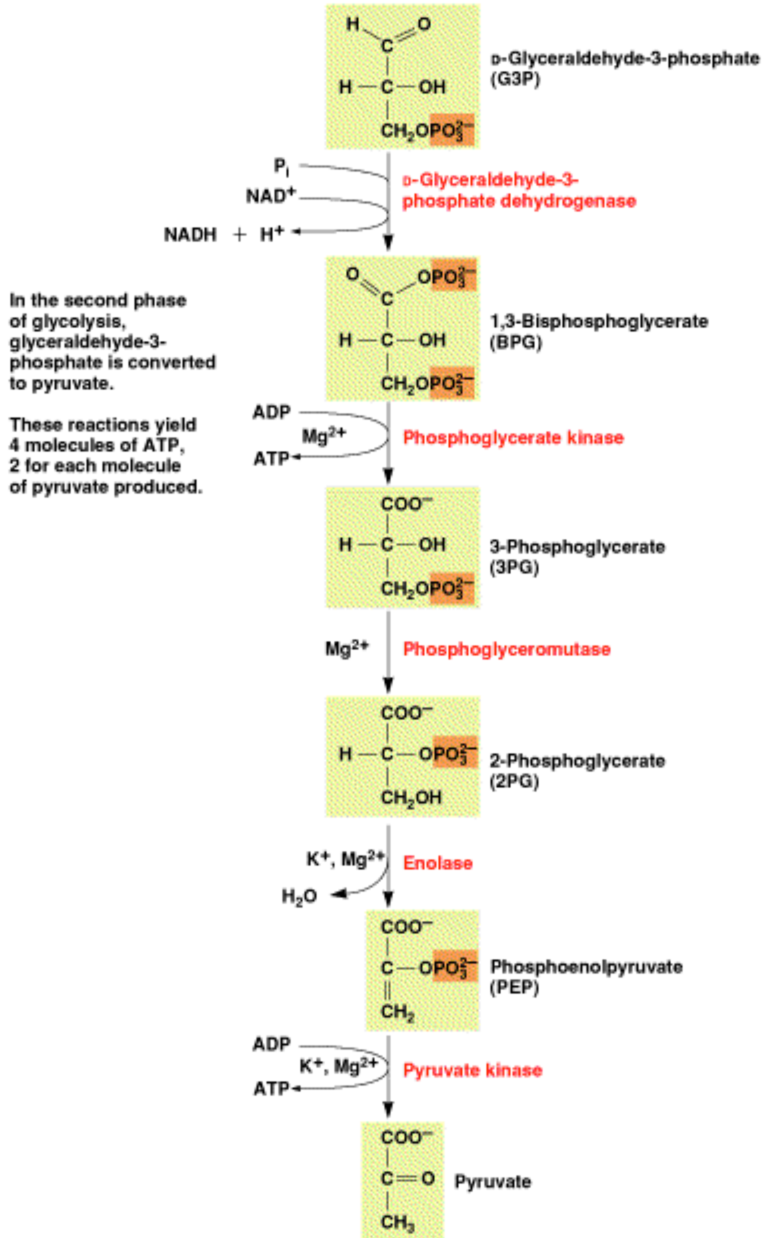


- **ENZYME: Triose phosphate isomerase**
  - **Isomerization – rearrangement reaction**
  - Isomerase enzyme
- **ATP COUNT:**
  - This step – 0
  - Overall count – -2
- **Not a regulatory step**

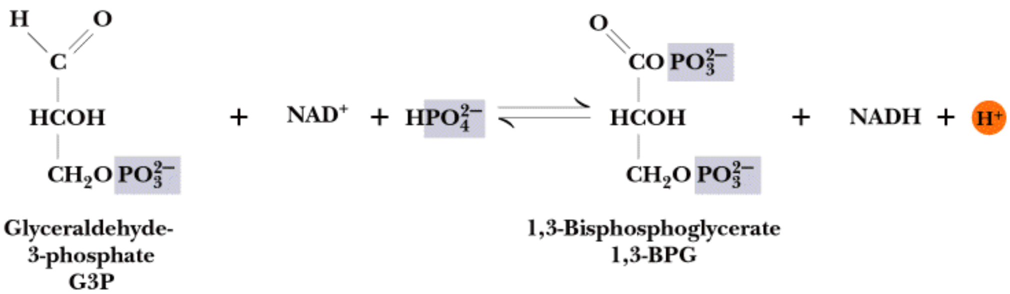
Through **1st 5 steps** (Investment Phase) we've **USED 2 ATP molecules**
Steps 6-10 → Dividend Phase where the investment pays off!!

Sum: Glucose + 2 ATP → 2 glyceraldehyde-3-phosphate +2 ADP + 2 Pi

Dividend Phase

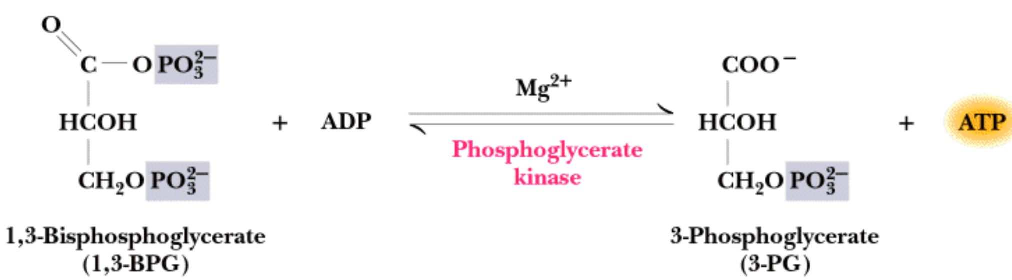


STEP 6: 2 Glyceraldehyde-3-phosphate to 2 1,3-bisphosphoglycerate



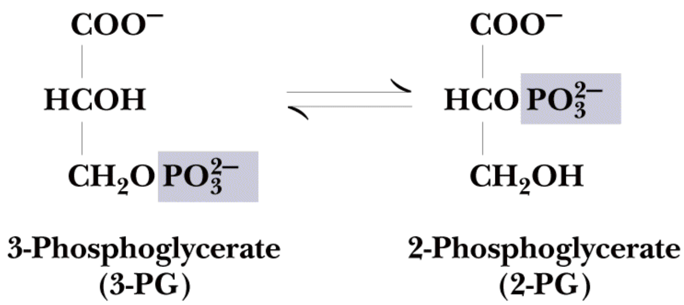
- **ENZYME: Glyceraldehyde-3-phosphate dehydrogenase**
  - **DEHYDROGENASE reaction**
  - Oxidation – Reduction enzymes (also called oxidoreductases)
  - Reactions generate either NADH, $FADH_2$ or NADPH
  - This reaction produces **NADH**
- **ATP COUNT:**
  - This step – 0
  - Overall count – -2
  - +2 NADH produced
- **Not a regulatory step**

STEP 7: (2) 1,3-bisphosphoglycerate to (2) 3-phosphoglycerate



- **ENZYME: Phosphoglycerate Kinase**
  - **Group Transfer reaction – KINASE reaction (same as 1 and 3)**
  - STEP WHERE ATP IS MADE!!
- **ATP COUNT:**
  - This step – +2
  - Overall count – -2 + 2 = 0
  - +2 NADH overall
- **Not a regulatory step**

STEP 8: (2) 3-phosphoglycerate to (2) 2-phosphoglycerate

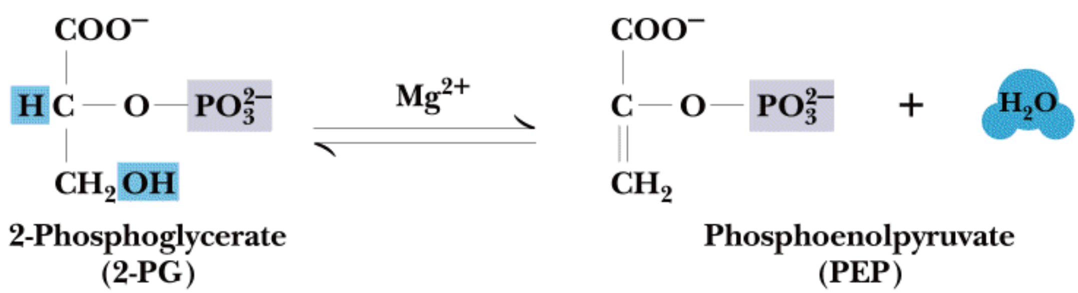


- **ENZYME: Phosphoglycerate Mutase**
  - **Isomerization – Rearrangement reaction – Mutase reaction (same as 2 and 5)**

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- **Not a regulatory step**

STEP 9: (2) 2-phosphoglycerate to (2) phosphoenolpyruvate

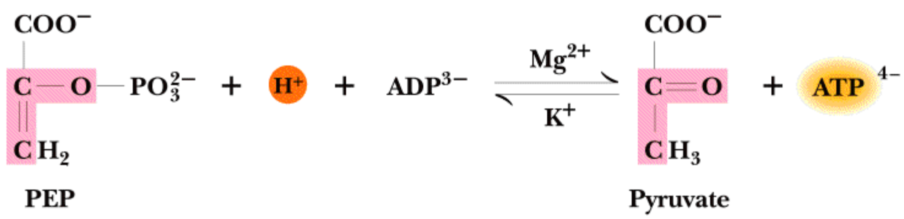


- **ENZYME: Enolase**
  - **Non-hydrolytic cleavage reaction (lyase)**

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- **Not a regulatory step**

STEP 10: (2) phosphoenolpyruvate to (2) pyruvate



$\Delta G°' = -31.7$ kJ/mol

- **ENZYME: Pyruvate Kinase**
    - **Group Transfer reaction – KINASE reaction (same as 1 and 3)**
    - STEP WHERE ATP IS MADE!!
- **ATP COUNT:**
    - This step – +2
    - Overall count – -2 + 2 + 2= 2
    - +2 NADH overall
- **This is a REGULATED step – Not Reversible**

GLYCOLYSIS ANIMATION:
http://www.northland.cc.mn.us/biology/Biology1111/animations/glycolysis.html

Table 15.2
The ATP and NADH balance sheet for glycolysis

Number[a]	Reaction per Glucose	ATP Change per Glucose[b]	NADH Change per Glucose[b]
1	Glucose → glucose-6-phosphate	−1	0
3	Fructose-6-phosphate → fructose-1,6-bisphosphate	−1	0
6	2 Glyceraldehyde-3-phosphate ⇌ 2 1,3-bisphosphoglycerate	0	+2
7	2 1,3-Bisphosphoglycerate ⇌ 2 3-phosphoglycerate	+2	0
10	2 Phosphoenolpyruvate → 2 pyruvate	+2	0
Total change		+2	+2

[a] The number corresponds to the reaction number in Table 15.1.
[b] A minus sign indicates loss of ATP by cleavage of a phosphoanhydride bond; a plus sign indicates formation of ATP (from ADP) or NADH (from NAD^+).

Table 15-2 Concepts in Biochemistry, 3/e

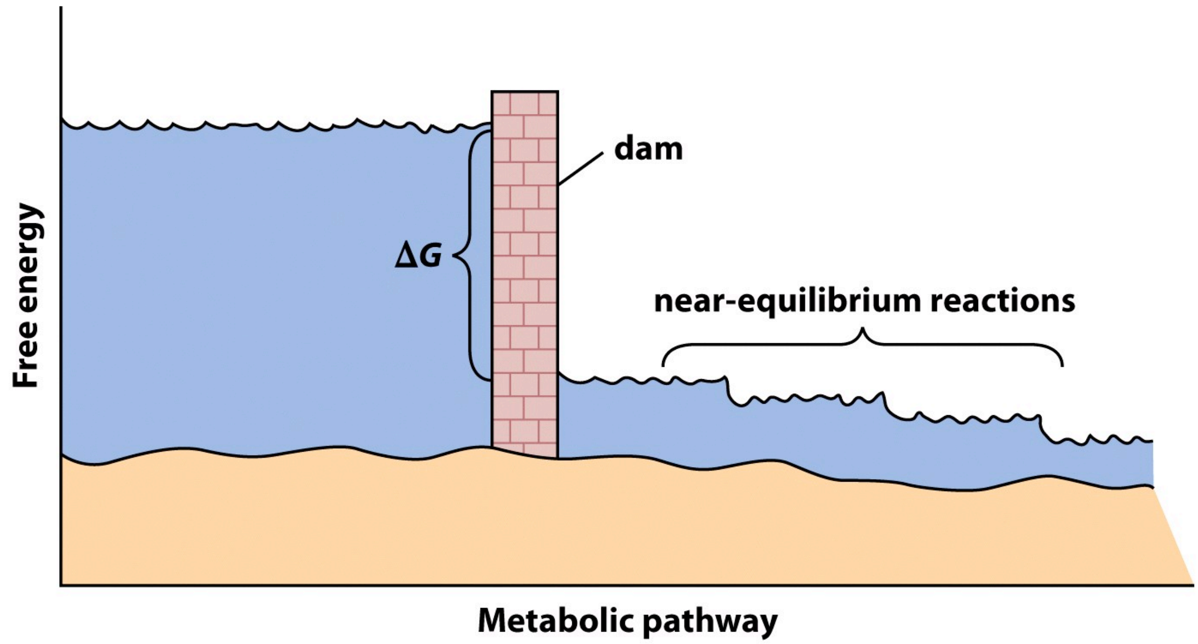
GLYCOLYSIS THERMODYNAMICS

Can regulate flux through a pathway by adjusting the rate of a reaction with a large free energy change.

- Increast the amount of enzyme
- Alter activity of the enzyme by small molecules

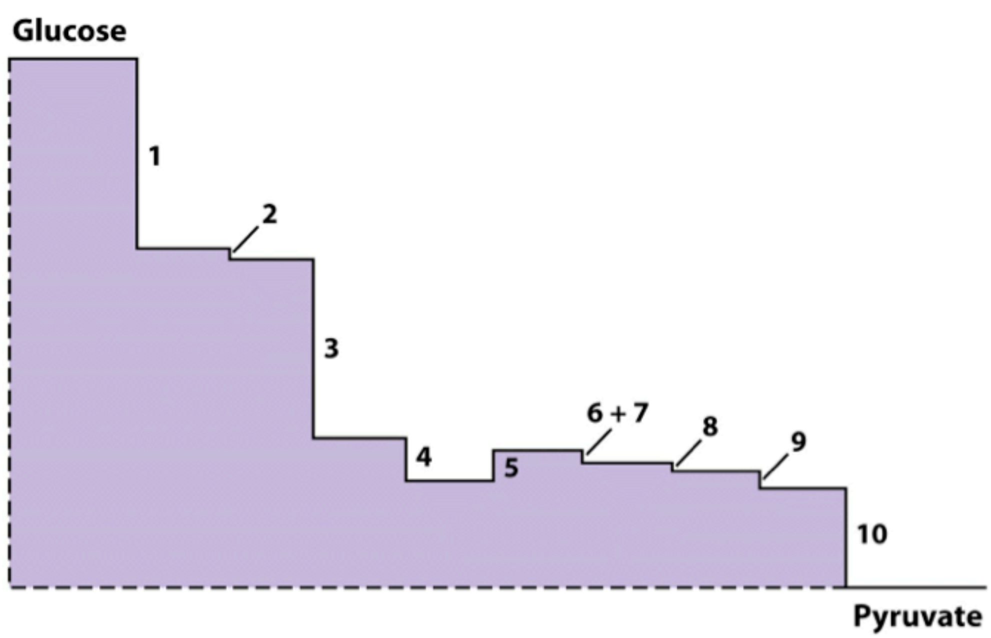
Three steps in glycolysis have large negative ΔG values (1, 3, 10)

Remaining steps are near equilibrium (ΔG ~ = 0)



As soon as the substrate has gotten past the "dam", the near-equilibrium reactions go with the flow, allowing the pathway intermediates to move toward the final product.

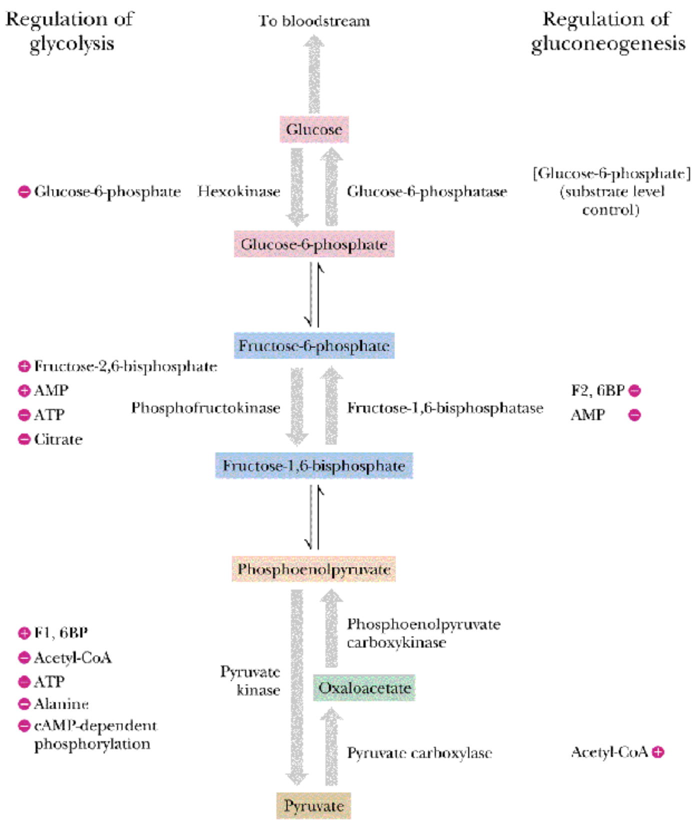
Usually multiple control points (dams) in a metabolic pathway.



The height of each step corresponds to its ΔG value in heart muscle glycolysis.
The numbers correspond to the glycolytic enzymes.

REGULATION OF GLYCOLYSIS

- **Glycolysis is a highly regulated process**
  - Need to maintain constant levels of energy in cells
  - Regulation UP and DOWN depends on the cell's need for ATP and NADH
  - Steps **2, 4-9** have ΔG°' values close to **zero**, therefore are essentially operating at **equilibrium**
    - Can go in either direction
    - These steps are common to the GLUCONEOGENESIS pathway
  - **Steps 1, 3 and 10 have large negative ΔG°' values (not at equilibrium) and are the sites of regulation.**



THREE KEY REGULATED STEPS

1. **Hexokinase** (Step #1)
   a. Regulates entry of free glucose into glycolysis
   b. Controlled by FEEDBACK INHIBITION
      i. Inhibited by product – glucose-6-phosphate
   c. NOT the committed step
   d. Regulates the concentration of glucose-6-phosphate

2. **Phosphofructokinase** (PFK) (Step #3)
   a. Catalyzes phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (FBP)
   b. **KEY REGULATORY POINT OF GLYCOLYSIS**
   c. Valve that controls glycolysis
   d. 1^{st} major committed step – can't go back
   e. PFK is **INACTIVE** when [ATP] cell is **HIGH**
      i. Makes good sense – when ATP is high, glycolysis no necessary so turned down at PFK
   f. If [AMP] (low energy precursor of ATP) HIGH, tells cell energy is LOW and to make more ATP
   g. Inhibited by **CITRATE** – physiological form of citric acid
      i. Citrate formed in TCA cycle from pyruvate
      ii. Therefore, if cellular [citrate] is sufficient, glycolysis is slowed
   h. <u>ACTIVATED</u> by fructose-2,6-bisphosphate (made when blood glucose conc. high)

3. **Pyruvate Kinase** (Step #10)
   a. Regulates formation of pyruvate from phosphoenolpyruvate
   b. Increase [ATP] inhibits pyruvate kinase and slows pyruvate formation

- Red blood cells depend on a constant energy supply to maintain structural integrity
  - Remember that they don't have nuclei or mitochondria
  - Therefore, **glycolysis is the primary source of ATP for red blood cells**
- If energy needs are not met, the RBC's can rupture (called hemolysis) and the blood loss called **hemolytic anemia**

- 2nd most common form of **hemolytic anemia** is due to deficiency in *pyruvate kinase*
  - Autosomal recessive trait (carriers have no disease)
  - Treated with transfusions and/or splenectomies
  - No simple treatment

Table 15.3

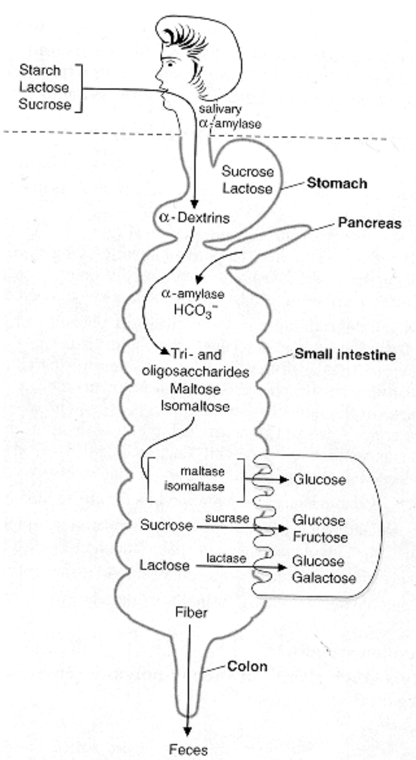
The irreversible reactions of glycolysis that are bypassed in gluconeogenesis

Number[a]	Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ/mol)
1	Glucose + ATP → glucose-6-phosphate + ADP	Hexokinase	−16.7
3	Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP	Phosphofructokinase	−14.2
10	Phosphoenolpyruvate + ADP → pyruvate + ATP	Pyruvate kinase	−31.4

[a] The number corresponds to the reaction number in Table 15.1.

Table 15-3 Concepts in Biochemistry, 3/e

ENTRY OF OTHER CARBOHYDRATES INTO GLYCOLYSIS:



1. Dietary Starch

a. Hydrolyzed in mouth by amylases to glucose monomers
b. Hydrolyzed in stomach by acid to glucose monomers
c. Glucose absorbed through intestinal walls to blood and transported
   i. 1/3 goes to skeletal muscle and heart
   ii. 1/3 goes to BRAIN – needs 100g glucose/day; can't use fatty acids
   iii. 1/3 goes to liver for storage as glycogen

2. Disaccharides:

a. Maltose → 2 glucose
b. Sucrose → fructose and glucose
c. Lactose → glucose and galactose

$$\text{maltose} + H_2O \xrightleftharpoons{\text{maltase}} 2\ \text{glucose}$$

$$\text{sucrose} + H_2O \xrightleftharpoons{\text{sucrase}} \text{fructose} + \text{glucose}$$

$$\text{lactose} + H_2O \xrightleftharpoons{\text{lactase}} \text{glucose} + \text{galactose}$$

Fructose and galactose enter glycolysis differently!

- **Fructose:**
  - In muscle, hexokinase phosphorylates fructose and enters pathway as fructose-6-phosphate. One step!
  - In liver, multiple steps needed.
    - Fructokinase phosphorylates at position 1
    - Aldolase cleavage
    - Additional phosphorylation
    - Enters as 1 molecule of DHAP that isomerizes to glyceraldehyde-3-phosphate
    - Glyceraldehyde product gets phosphorylated and then enters glycolysis as well
    - All 6 carbons enter as two molecules.

- **Galactose:**
  - C4 epimer of glucose
  - Requires 5 reactions to transform it to *glucose-6-phosphate* where it can enter glycolytic pathway

- **Glycerol:**
  - Released during degradation of TAG's
  - 2 Reactions:
    - Phosphoryl transfer
    - Oxidation
  - Turns glycerol into dihydroxyacetone phosphate which isomerizes in glycolysis to glyceraldehyde-3-phosphate

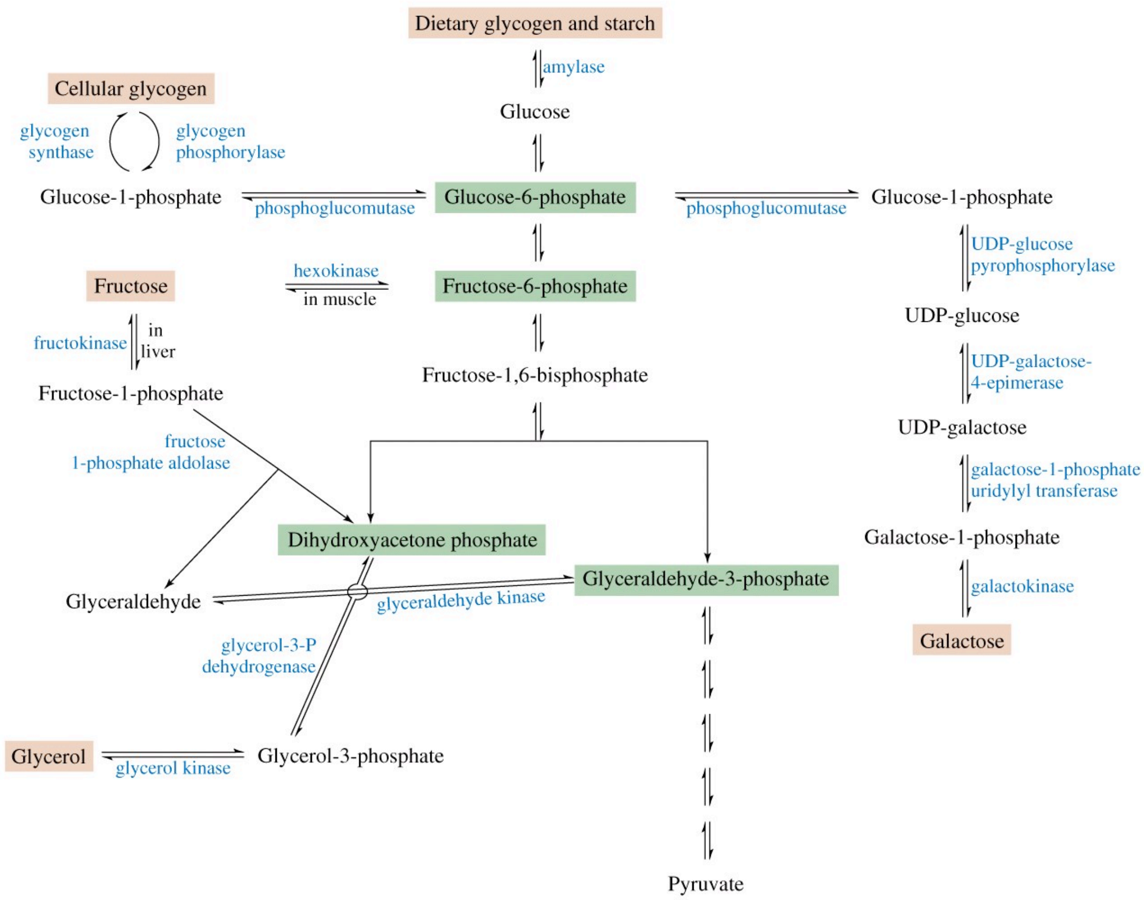


Figure 15-3 Concepts in Biochemistry, 3/e