GLYCOLYSIS

- All cells carry out glycolysis
- The entire process occurs in cytoplasm

Glucose + 2 ADP + 2 NAD^+ + 2 P_i -> 2 pyruvate + 2 ATP + 2 NADH + 2H^+ + 2H_2O

Unless NAD^+ is regenerated, glycolysis will stop

NAD^+ regeneration occurs via two mechanisms depending on the cell type and the availability of oxygen

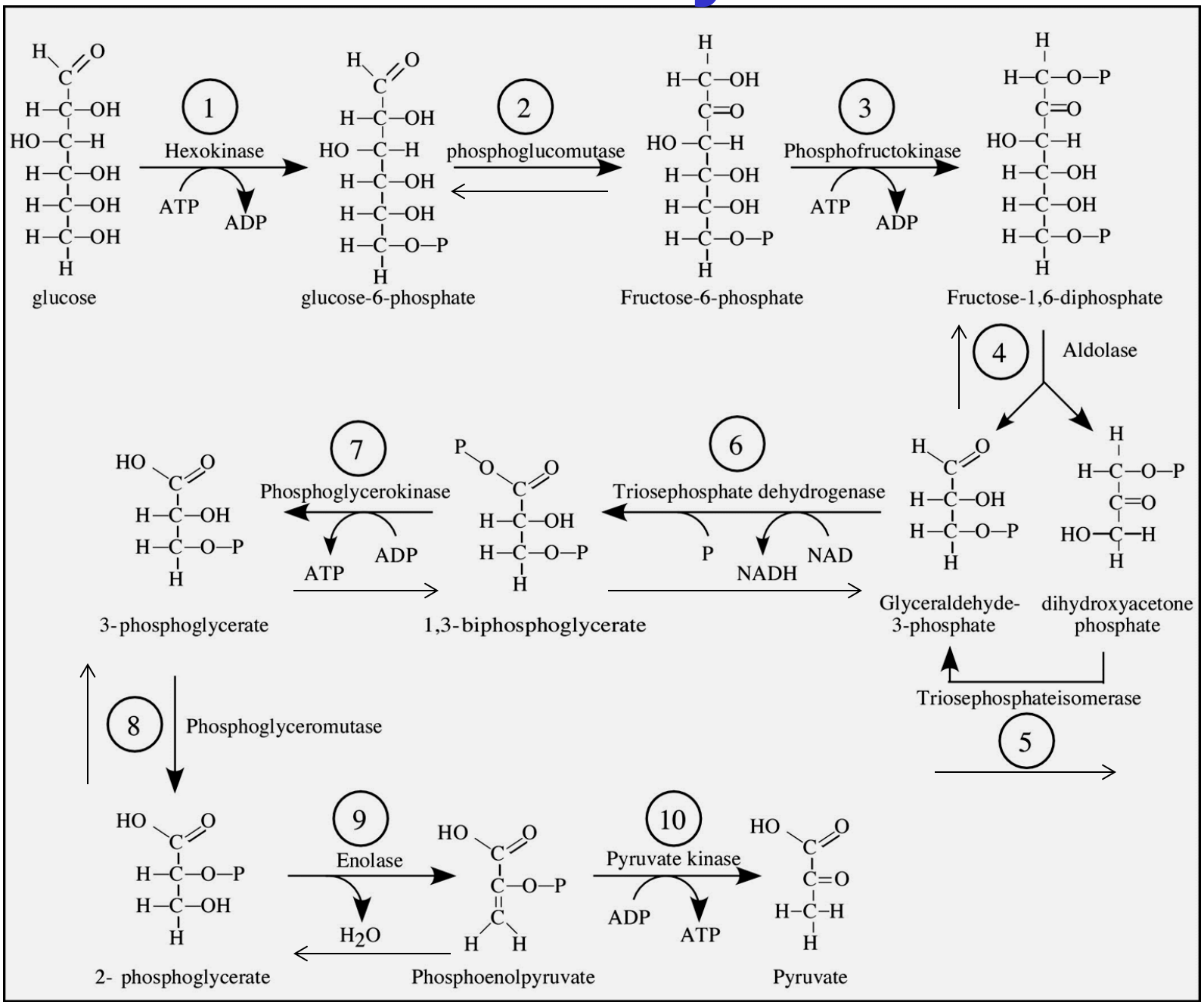
In mitochondria-containing cells and in the presence of oxygen, the regeneration occurs via electron transport chain. Then pyuvate is the end product of glycolysis, and the pyruvate gets oxidized in mitochondria

When oxygen is not available, even in mitochondria-containing cells, NAD^+ cannot be regenerated in mitochondria. Then the only way to regenerate NAD^+ and keep glycolysis going is to convert pyruvate into lactate using NADH. This process occurs in cytoplasm and also regenerates NAD^+

In erythrocytes (RBC), there is no mitochondria. But these cells entirely depend on glycolysis for energy. Therefore, these cells produce lactate in glycolysis all the time.

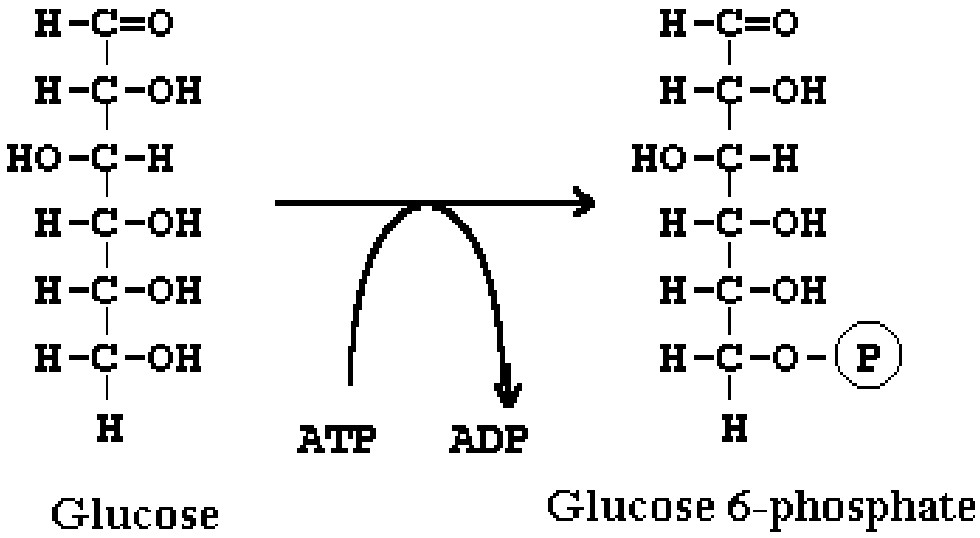
Tumor cells also use this method to keep glycolysis going. They produce lactate from pyruvate as a mechanism to regenerate NAD^+.

Pathway


glucose
1
Hexokinase
ATP
ADP
glucose-6-phosphate
2
phosphoglucomutase
Fructose-6-phosphate
3
Phosphofructokinase
ATP
ADP
Fructose-1,6-diphosphate
4
Aldolase
Glyceraldehyde-3-phosphate
dihydroxyacetone phosphate
Triosephosphateisomerase
5
6
Triosephosphate dehydrogenase
P
NADH
NAD
1,3-biphosphoglycerate
7
Phosphoglycerokinase
ATP
ADP
3-phosphoglycerate
8
Phosphoglyceromutase
2- phosphoglycerate
9
Enolase
H_2O
Phosphoenolpyruvate
10
Pyruvate kinase
ADP
ATP
Pyruvate

Rx 1: Hexokinase/Glucokinase

- Irreversible
- ATP is consumed here



Different Hexokinase Isozymes

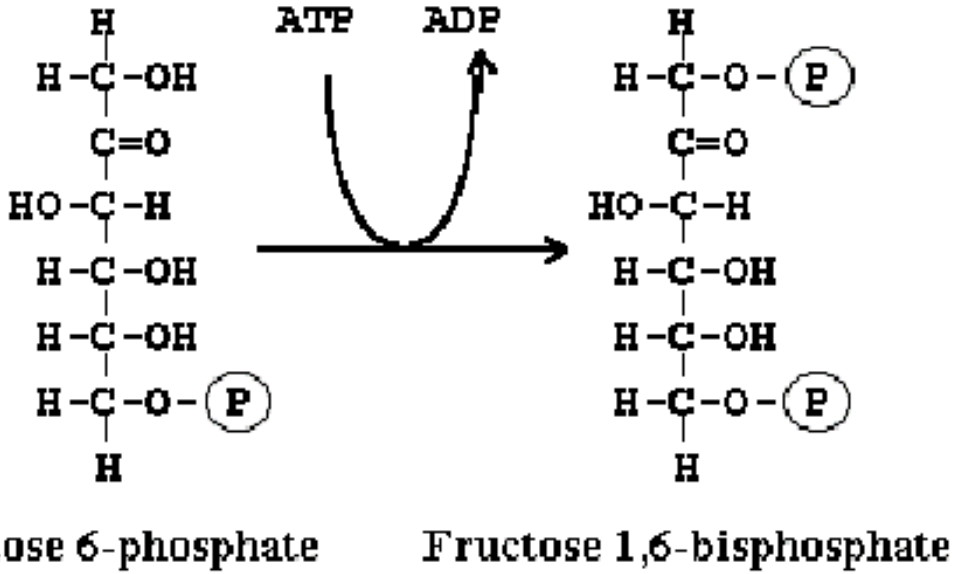
- Two major forms: hexokinase (all cells) & glucokinase (liver and pancreas)
- K_m for hexokinase is 0.1 mM; It is called a high-affinity/low-capacity type.
- Cells have 4-5 mM glucose; under these conditions, hexokinase functions at a fully saturated level; changes in blood levels of glucose are not sensed or detected by this enzyme.
- Its activity is subject to product inhibition; glucose-6-phosphate inhibits this enzyme.

Glucokinase

- K_m for glucokinase is 10 mM; It is called a low-affinity/high-capacity type; It becomes significantly active only when cells have lots of glucose.
- Glucokinase functions when blood glucose levels are high to enhance glycolysis and glycogen storage in the liver.
- Its activity changes in proportion to blood glucose levels
- In pancreas, ATP produced in glycolysis is responsible for insulin secretion; therefore, glycolysis rate must change in proportion to blood glucose levels to co-ordinate insulin secretion.
- Higher blood glucose ➔Higher glucokinase activity ➔Higher ATP production in glycolysis ➔Higher insulin secretion
- Glucokinase is not subject to product inhibition; glucose-6-phosphate does not inhibit this enzyme
- This enzyme is inducible by insulin

Rx 3: Phosphofructokinase-1 (PFK-1)

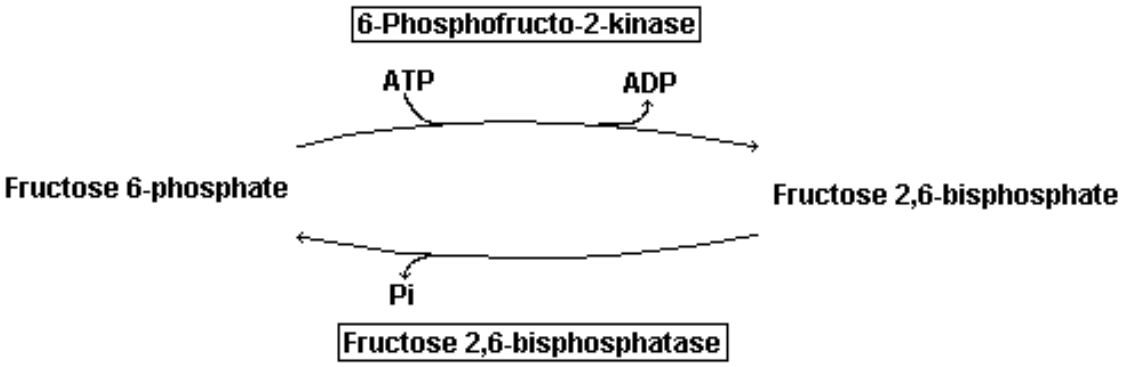
- Irreversible
- PFK-1 is highly regulated
- PFK-1 has many allosteric activators and inhibitors
- Second stage where ATP is consumed

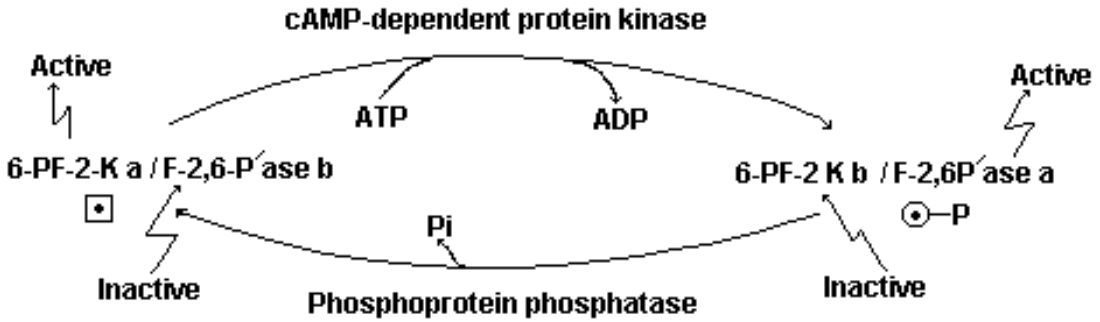


PFK-1 is highly regulated

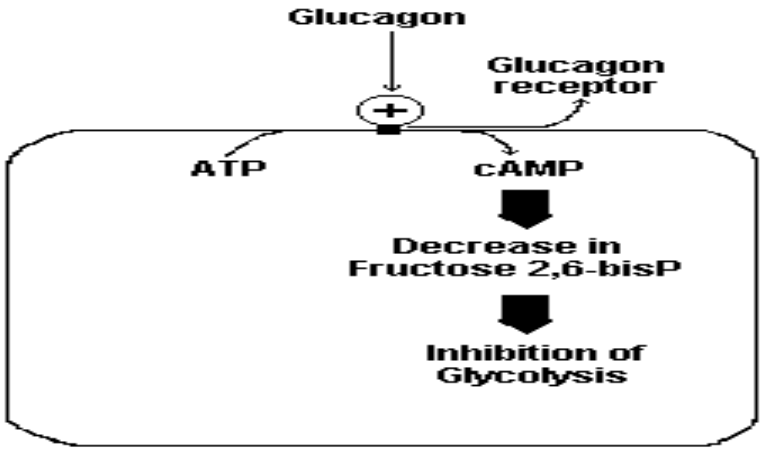
- PFK-1 is an allosteric enzyme
- ATP and citrate are allosteric inhibitors
- AMP is an allosteric activator
- Fructose-2,6-bisphosphate is an important allosteric activator
- F2,6BP is not an intermediate in glycolysis; it is also not the product of PFK-1

Fructose-2,6-bisphosphate synthesis and glycolysis regulation





In Diabetes, Insulin↓
Glucagon↑



cAMP and F2,6BP

- F2,6BP is synthesized from F6P by phosphofructokinse-2 (PFK-2)
- F2,6BP is degraded by F2,6BPase
- PFK-2 and F2,6BPase reside on the same protein
- PFK-2/F2,6BPase is regulated by cAMP-dependent protein kinase A (PKA)
- Regulation occurs differentially in liver, heart, and skeletal muscle because different isoenzymes are expressed differentially in these three tissues.

Relationship between cAMP and F2,6BP

Liver

- There is a reciprocal relationship between cAMP and F2,6BP in liver; higher cAMP, the lower F2,6BP
- When glucagon levels are high in blood as in uncontrolled diabetes, cAMP levels go up in liver; this activates protein kinase A, which then phosphorylates the bifunctional enzyme PFK-2/F2,6BPase; this inactivates PFK-2 but activates F2,6BPase; the result is a decrease in F2,6BP
- This leads to decreased activity of PFK-1, thus decreasing glycolysis and glucose breakdown
- Glucagon is a hyperglycemic hormone; it prevents breakdown of glucose in liver.

Relationship between cAMP and F2,6BP

Heart

There is a direct relationship between cAMP and F2,6BP in liver; higher cAMP, the higher F2,6BP

When epinephrine (adrenaline) levels are high in blood as in stressful condition (flight or fight situation), cAMP levels go up in heart; this activates protein kinase A, which then phosphorylates the bifunctional enzyme PFK-2/F2,6BPase; this activates PFK-2 but has not effect on F2,6BPase; the result is an increase in F2,6BP

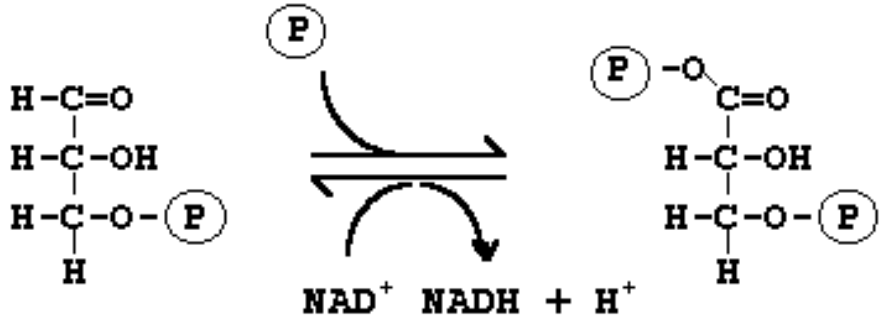
This leads to increased activity of PFK-1, thus increasing glycolysis and glucose breakdown in heart, thus increasing ATP and heart function

Epinephrine enhances cardiac activity

This differential effect of cAMP in heart versus liver is possible because different isoforms of the enzyme are expressed in these two tissues

Rx 6: Glyceraldehyde-3P-Dehydrogenase

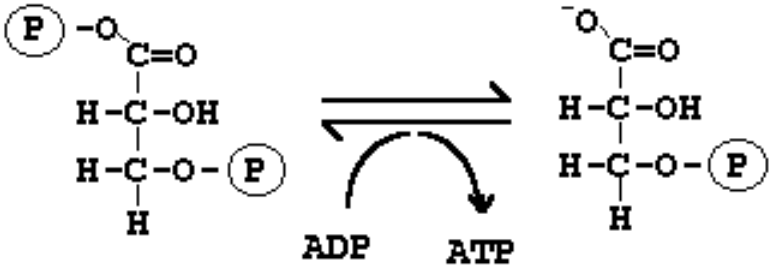
- Reversible
- Pi is used as phosphate donor
- 1,3 BPG is a high energy molecule.
- C1 phosphoryl group has high group transfer potential, used to phosphorylate ADP to ATP in next step of glycolysis
- Arsenate can replace phosphate in rxn (results in lower ATP)
- NADH generated in this reaction



Glyceraldehyde 3-phosphate 1,3-Bisphosphoglycerate

Rx 7: Phosphoglycerate Kinase

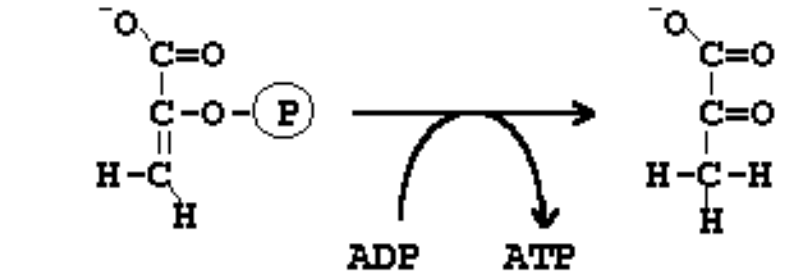
- Reversible
- ATP synthesis from a high-energy phosphate
- This is referred to as "substrate-level phosphorylation”



1,3-Bisphosphoglycerate 3-Phosphglycerate

Rx 10: Pyruvate Kinase

- Substrate-level phosphorylation generates second ATP
- Allosterically activated by AMP, F-1,6-bisP
- Allosterically inhibited by ATP



cAMP and pyruvate kinase in liver

- cAMP inhibits pyruvate kinase activity in liver by Protein kinase A-dependent phosphorylation
- When glucagon levels go up in blood as in uncontrolled diabetes, cAMP levels in liver increase, resulting in activation of PKA.
- This leads to phosphorylation of pyruvate kinase, thus inhibiting the enzyme activity
- The end result is a decrease in glycolysis
- Thus, glucagon prevents breakdown of glucose, thus increasing the circulating levels of glucose

Uncontrolled diabetes and liver

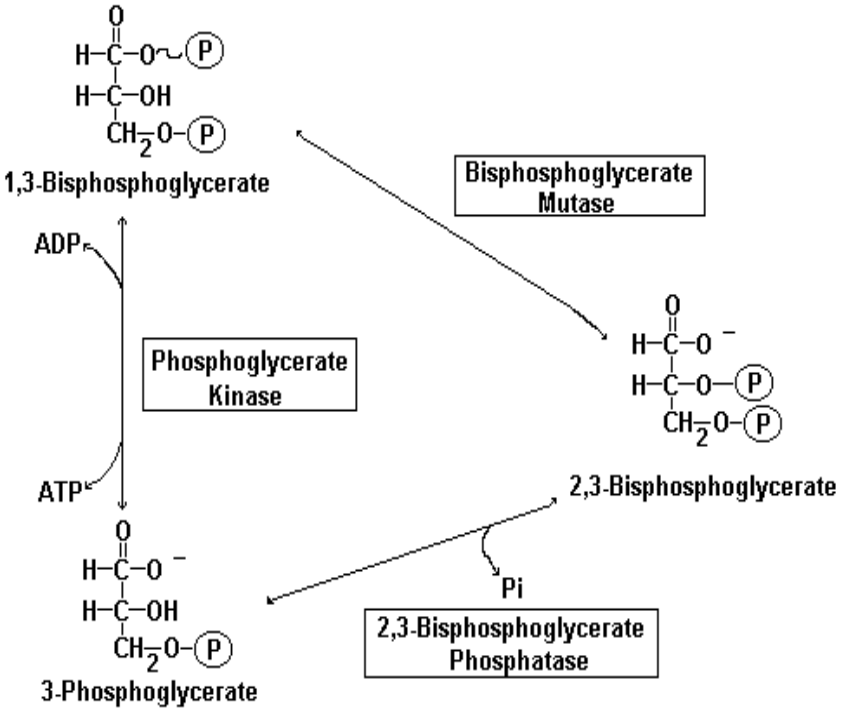
- In uncontrolled diabetes, insulin levels are low and glucagon levels are high
- cAMP levels go up in liver, thus activating PKA
- PFK-2/F2,6BPase is phosphorylated, resulting in decreased F2,6BP
- PFK-1 activity goes down, thus decreasing the rate of glycolysis
- PKA also phosphorylates pyruvate kinase, thus inhibiting the enzyme and also decreasing glycolysis
- Since insulin levels are low, there is decreased induction of glucokinase, the isoenzyme epecifically expressed in liver
- This also decreases glycolysis
- The end result is prevention of glucose breakdown, thus increasing the levels of glucose in blood
- Hence hyperglycemia in diabetes

Pyruvate kinase deficiency

- Glycolysis is the only source of ATP in erythrocytes
- Genetic defects in pyruvate kinase decrease glycolysis and hence decrease ATP in RBC, thus leading to hemolysis
- Increased hemolysis causes anemia and also jaundice
- Increased risk of gallstones (bilirubin stones; not cholesterol stones)
- Any defect in any of the glycolytic enzymes will lead to hemolytic anemia, but genetic mutations in pyruvate kinase represent the most prevalent genetic causes of hemolytic anemia
- No Heinz bodies (precipitated hemoglobin stuck to the cytoplasmic side of RBC plasma membrane) in blood smear; this is in contrast to hemolytic anemia seen in patients with mutations in glucose-6-phosphate dehydrogenase, an enzyme associated with pentose phosphate pathway

2,3-Bisphosphoglycerate is made by from 1,3-BPG as a side reaction

- Erythrocytes contain high level of this side reaction; they contain 4-5 mM 2,3-BPG
- 2,3-BPG acts to maintain hemoglobin in low oxygen affinity form
- The levels of 2,3-BPG in RBC increase at high altitude and low oxygen pressure



Enzyme defects in glycolysis and 2,3BPG

- Loss of function of pyruvate kinase increases 2,3BPG levels in RBC
- This results in shifting of the oxygen-binding to hemoglobin curve to the right

- If the enzyme defect lies upstream of 1,3-BPG in the glycolysis pathway, hemolysis will occur, but the levels of 2,3BPG will decrease, thus shifting the oxygen-binding to hemoglobin curve to the left