# Lecture 3 - Glycolysis and Gluconeogenesis

Chem 454: Regulatory Mechanisms in Biochemistry
University of Wisconsin-Eau Claire

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## Introduction

Glycolysis converts glucose ($C_6H_{12}O_6$) molecules to two molecules of pyruvic acid ($C_3H_4O_3$).

- Pyruvic acid is more oxidized than glucose
- The energy released from the oxidation is used to create 2 molecules of ATP from 2 ADP and 2 $P_i$
- This is an **anaerobic** process.
- Under anaerobic conditions the pyruvic acid can be **fermented** to lactic acid or to ethanol plus $CO_2$.
- Under **aerobic** conditions, glucose is oxidized all the way to $C0_2$ and $H_2O$.

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## Introduction

Glucose can also be synthesized from molecules such as pyruvic acid or lactic acid.

- This process is called **gluconeogenesis**.

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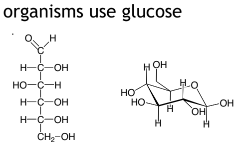
## Introduction

- Glucose is an important fuel for most organisms.
  - In mammals, glucose is the preferred fuel source for the brain and the only fuel source for red blood cells.
  - Almost all organisms use glucose



D–Glucose β–D–Glucose

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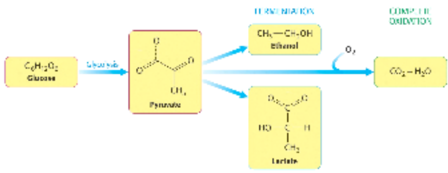
## Introduction

Fermentations provide usable energy in absence of oxygen.



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## Introduction

Obligate anaerobes

**TABLE 16.2 Examples of pathogenic obligate anaerobes**

| Bacterium | Results of infection |
|---|---|
| *Clostridium tetani* | Tetanus (lockjaw) |
| *Clostridium botulinum* | Botulism (an especially severe type of food poisoning) |
| *Clostridium perfringens* | Gas gangrene (gas is produced as an end point of the fermentation, distorting and destroying the tissue) |
| *Bartonella hensela* | Cat scratch fever (flulike symptoms) |
| *Bacteroides fragilis* | Abdominal, pelvic, pulmonary, and blood infections |



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## 1. Glycolysis is Energy Conversion

Glycolysis is an energy-conversion pathway in many organisms.

- The glycolytic pathway is common to virtually all organisms
- Both eukaryotes and prokaryotes
- In eukaryotes, it occurs in the cytosol



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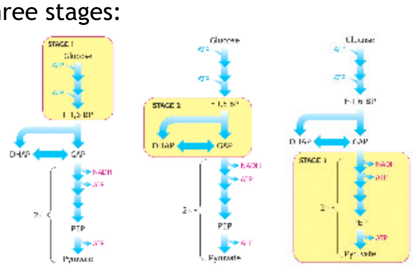
## 1. Glycolysis is Energy Conversion

The glycolytic pathway is considered in three stages:



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## 1. Glycolysis is Energy Conversion

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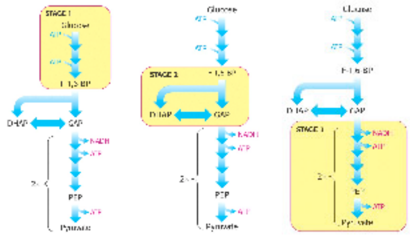


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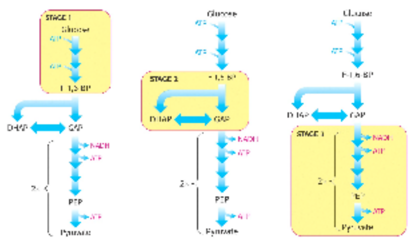
## 1. Glycolysis is Energy Conversion

The glycolytic pathway is considered in three stages:

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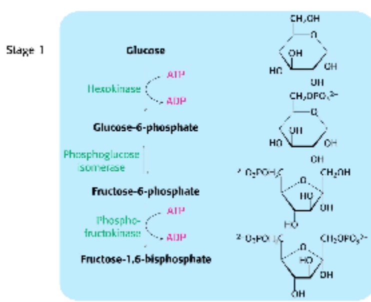
## 1. Stage 1

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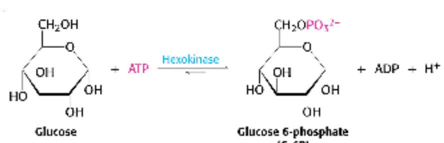
## 1.1. Hexokinase

Hexokinase traps glucose in the cell and begins glycolysis.

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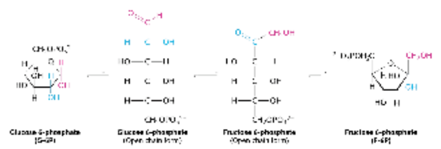
## 1.2 Phosphoglucose Isomerase

The formation of fructose 1,6-bisphosphate from glucose 6-phosphate

- Phosphoglucose isomerase



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## 1.2 Phosphofructokinase

The formation of fructose 1,6-bisphosphate from glucose 6-phosphate

- Phosphofructose kinase


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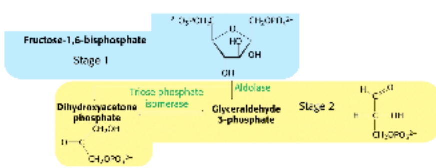
## 1. Stage 2

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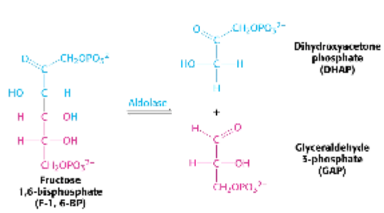
## 1.3. Aldolase

The six-carbon sugar is cleaved into two three-carbon fragments by aldolase.

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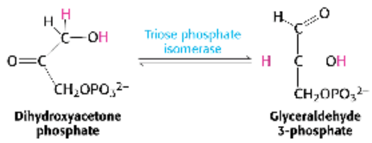
## 1.4. Triose Phosphate Isomerase

Triose phosphate isomerase salvages a three-carbon fragments





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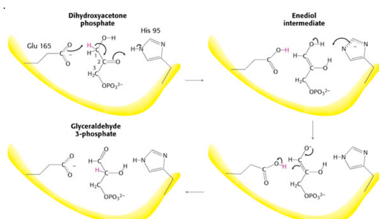
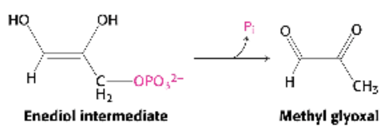







## 1.4. Triose Phosphate Isomerase

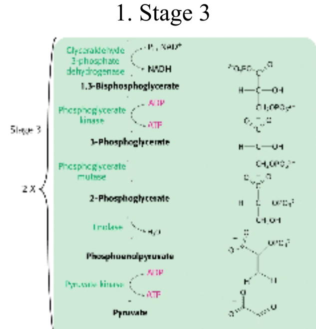
Triose phosphate isomerase is an example of a kinetically perfect enzyme.





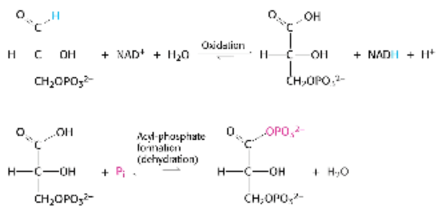



## 1. Stage 3



# 1.5. Glyceraldehyde 3-Phosphate Dehydrogenase

Energy transformation: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate.

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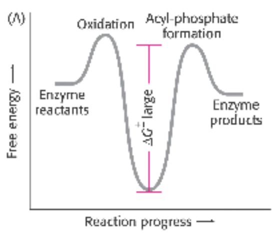


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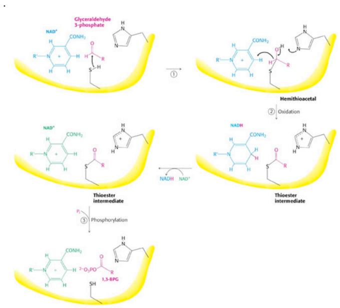
# 1.5. Glyceraldehyde 3-Phosphate Dehydrogenase



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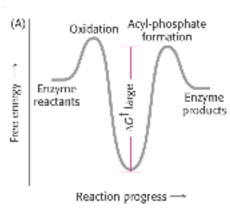
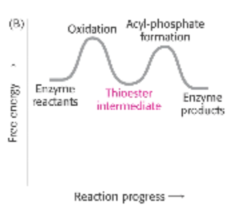
# 1.5. Glyceraldehyde 3-Phosphate Dehydrogenase

The enzyme-bound thioester intermediate reduces the activation energy for the second reaction:

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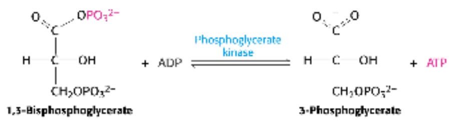
## 1.6. Phosphoglycerate Kinase

The acyl phosphate in 1,3-bisphosphoglycerate has a high enough phosphoryl transfer potential to phosphorylate ADP to produce ATP:

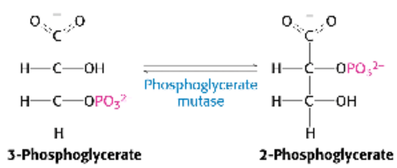





## 1.7. Phosphoglycerate Mutase

The next two reactions convert the remaining phosphate ester into a phosphate having a high phosphoryl transfer potential

- The first is an isomerization reaction

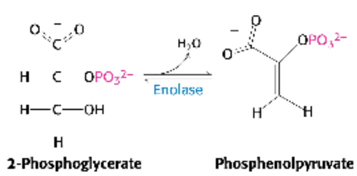





## 1.7. Enolase

The next two reactions convert the remaining phosphate ester into a phosphate having a high phosphoryl transfer potential

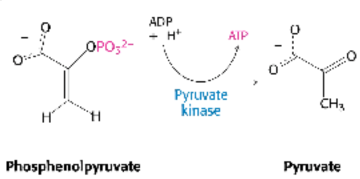
- The second is a dehydration (lyase) reaction





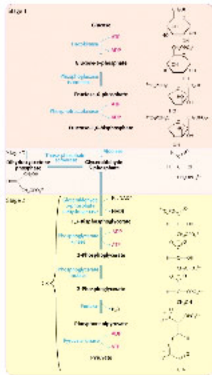

## 1.7. Pyruvate Kinase

The final reaction in the glycolytic pathway transfers the phosphate from phosphoenolpyruvate to ADP to produce ATP:



## 1.8 The Net Reaction

$$\text{Glucose} + 2\ NAD^+ + 2\ ADP + 2\ P_i \longrightarrow \text{Pyruvate} + 2\ NADH + 2\ H^+ + 2\ ATP$$

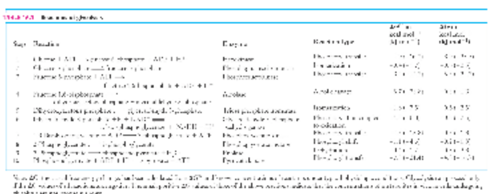






## 1.8. The Net Reaction

Summary of the reactions in the glycolytic pathway:







## 1.9.Maintaining Redox Balance

There is a problem:

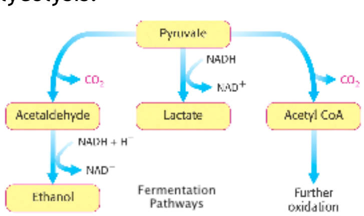
- There are only catalytic quantities of $NAD^+$ in the cell.
- In order to continue to use glycolysis to generate ATP, there needs to be some means of reoxidizing the $NADH + H^+$ that is produced in glycolysis





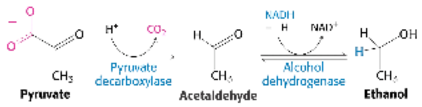
## 1.9.Maintaining Redox Balance

The solution to this problem lies in what happens to the pyruvate that is produced in glycolysis:



## 1.9.Maintaining Redox Balance

Ethanol fermentation is use by yeast and produces ethanol and $CO_2$.

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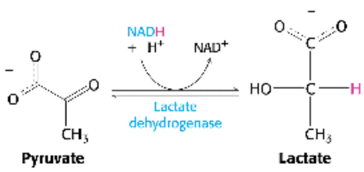
## 1.9.Maintaining Redox Balance

Lactic acid fermentation is use by bacteria and human muscles and produces lactate.

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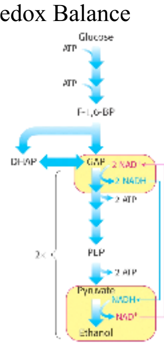
## 1.9.Maintaining Redox Balance

The fermentation pathways restore the redox balance:

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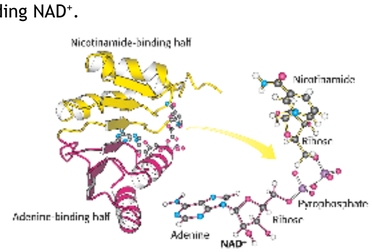
## 1.10 NAD$^+$ Binding

All three of the dehydrogenase in glycolysis and the fermentation pathways share a common domain for binding NAD$^+$.

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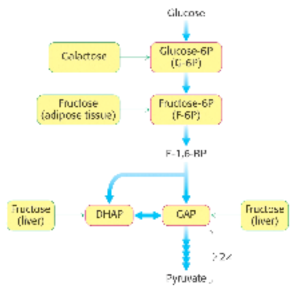
## 1.11 Other Points of Entry

The entry of fructose and galactose into glycolysis.

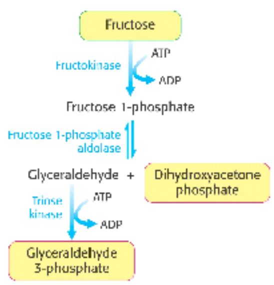







## 1.11 Other Points of Entry

Fructose is phosphorylated to fructose 1-phosphate in the liver.

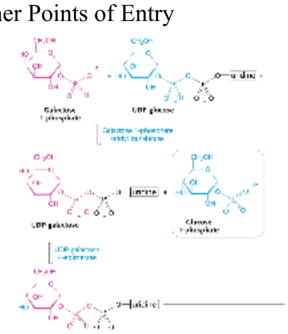







## 1.11 Other Points of Entry

Galactose is urdidylated before epimerization to UDP-glucose:

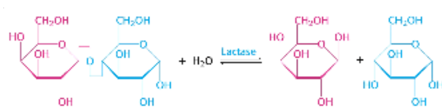






## 1.12 Lactose Intolerance

Many adults are intolerant of milk because they are deficient in lactase



## 1.13 Galactose is Highly Toxic

- Disruption of galactose metabolism is called *galactosemia*.
  - Usually due to loss of uridyl transferase activity
- Symptoms include
  - Failure to thrive infants
  - Enlarged liver and jaundice, sometimes cirrhosis
  - Cataracts
  - Mental retardation





## 2. Control of Glycolysis

Two major needs of the the cell influence the flow of material from glucose to pyruvate:

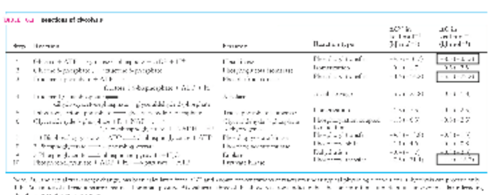
- The need for ATP (energy)
- The need for building blocks for biosynthesis





## 2. Control of Glycolysis

In metabolic pathways, control is focused on those steps in the pathway that are irreversible.







## 2. Control of Glycolysis

The different levels of control have different response times:

| Level of Control | Response Time |
|---|---|
| Allosteric | milleseconds |
| Phosphorylation | seconds |
| Transcriptional | hours |

## 2.1 Phosphofructokinase

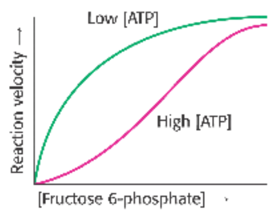
Phosphofructokinase is the key enzyme in the control of glycolysis.






## 2.1 Phosphofructokinase

Allosteric regulation of phosphofructo-kinase by ATP

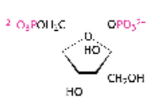


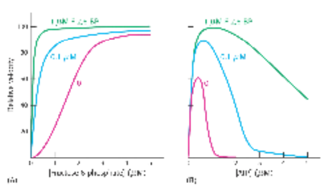





## 2.1 Phosphofructokinase

Phosphofructokinase is also regulated by fructose 2,6-bisphosphate:



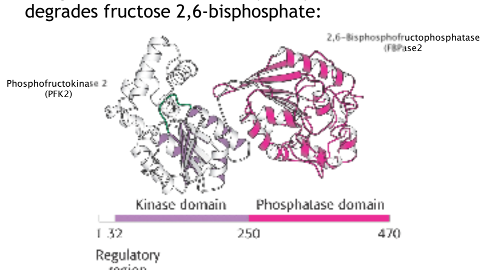







## 2.2. Fructose 2,6-bisphosphate

A regulated bifunctional enzyme synthesizes and degrades fructose 2,6-bisphosphate:



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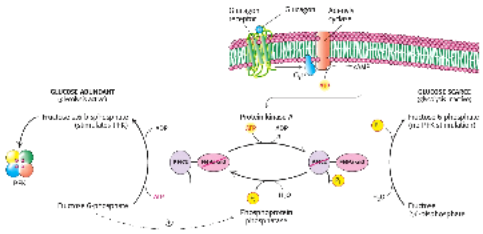

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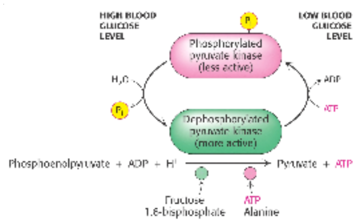
## 2.3 Hexokinase and Pyruvate Kinase

Hexokinase and pyruvate kinase also set the pace of glycolysis.

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## 2.4. Glucose Transporters

A family of transporters enables glucose to enter and leave animal cells.

TABLE 16.4 Family of glucose transporters

| Name | Tissue location | $K_m$ | Comments |
|---|---|---|---|
| GLUT1 | All mammalian tissues | 1 mM | Basal glucose uptake |
| GLUT2 | Liver and pancreatic β cells | 15–20 mM | In the pancreas, plays a role in regulation of insulin. In the liver, removes excess glucose from the blood |
| GLUT3 | All mammalian tissues | 1 mM | Basal glucose uptake |
| GLUT4 | Muscle and fat cells | 5 mM | Amount in muscle plasma membrane increases with endurance training |
| GLUT5 | Small intestine | — | Primarily a fructose transporter |

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## 3. Gluconeogenesis

Glucose can be synthesized from noncarbohydrate precursors.

- The brain has a strong preference for glucose, while the red blood cells have and absolute requirement for glucose.
- The brain needs 120 g of glucose/day
- The liver has about a 190 g store of glucose as glycogen. (About a 1 day's supply)
- Glucose can be synthesized in the liver from pyruvate, glycerol and amino acids.

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## 3.1. Gluconeogenesis

Gluconeogenesis is not the reverse of glycolysis.

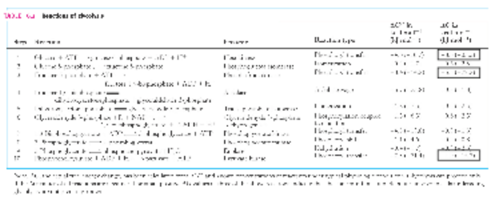
2 Pyruvate + 2 ATP + 2 NADH + 2 $H^+$ + 2 H2O
→ Glucose + 2 ADP + 2 Pi + 2 $NAD^+$

- The G°' for this reaction is +20 kcal/mol



## 3.1. Gluconeogenesis

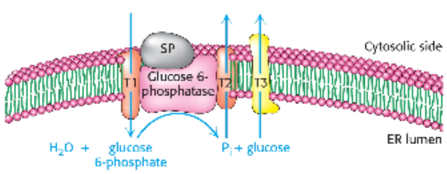
The three kinase reactions are the ones with the greatest positive free energies in the reverse directions





## 3.1. Gluconeogenesis

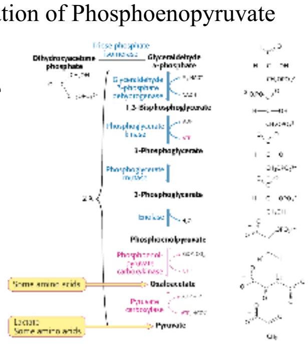
The hexokinase and phosphofructokinase reactions can be reversed simply with a phosphatase





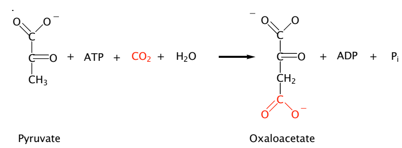

## 3.2. Formation of Phosphoenopyruvate

Reversing the pyruvate kinase reaction is not as easily done



## 3.2. Formation of Phosphoenopyruvate

The conversion of pyruvate into phosphoenolpyruvate begins with the formation of oxaloacetate.

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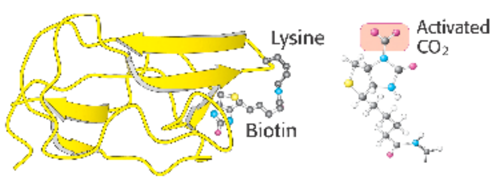
## 3.2. Formation of Phosphoenolpyruvate

Pyruvate kinase uses the biotin cofactor to activate the $CO_2$

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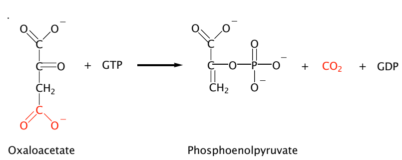
## 3.2. Formation of Phosphoenolpyruvate

The formation of phosphoenolpyruvate from oxaloacetate is driven both by the hydrolysis of GTP and a decarboxylation

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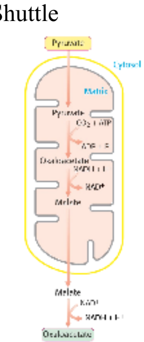
## 3.3. Oxaloacetate Shuttle

Oxaloacetate is synthesized in the mitochondria and is shuttled into the cytosol where it is converted into phosphoenolpyruvate



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## 3.6. “High-Energy” Phosphate Bonds

Six high-energy phosphate bonds are spent in synthesizing glucose from pyruvate.

Gluconeogenesis:

2 Pyruvate + 4 ATP + 2GTP + 2 NADH + 2 $H^+$ + 6 H2O → Glucose + 4 ADP + 2 GDP + 6 Pi + 2 $NAD^+$

$\Delta G°' = -9$ kcal/mol

Reverse of Glycolysis:

2 Pyruvate + 2 ATP + 2 NADH + 2 $H^+$ + 2 H2O → Glucose + 2 ADP + 2 Pi + 2 $NAD^+$

$\Delta G°' = +20$ kcal/mol

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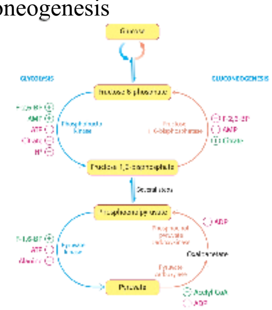
## 4. Regulation of Glycolysis and Gluconeogenesis

Reciprocal regulation of glycolysis and gluconeogenesis in the liver



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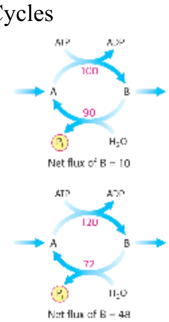
## 4.1. Substrate Cycles

Substrate cycles amplify metabolic signals and produce heat.

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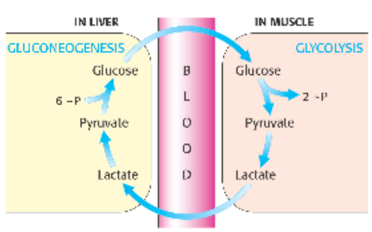
## 4.2. Lactate and Alanine

Lactate and alanine formed by contracting muscle are used by other organs

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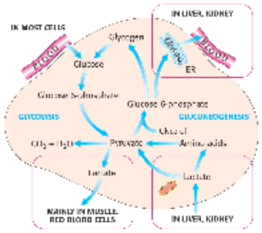
## 4.2. Lactate and Alanine

Lactate and alanine formed by contracting muscle are used by other organs








## 4.3. Evolution of Glycolysis and Gluconeogenesis

- Glycolysis and Gluconeogenesis are evolutionarily intertwined.