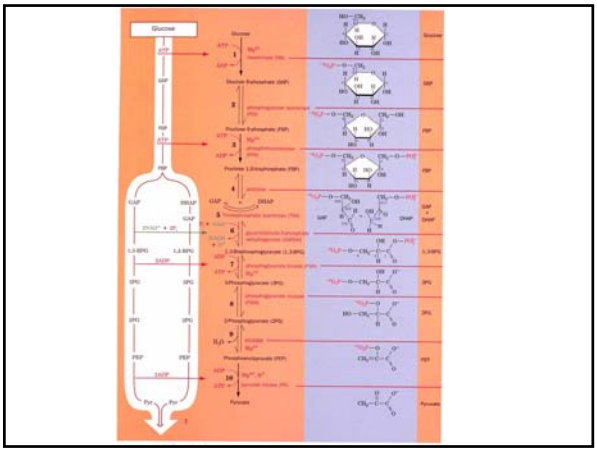
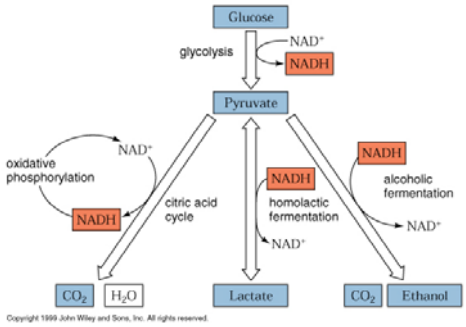
Glycolysis III

11/10/09

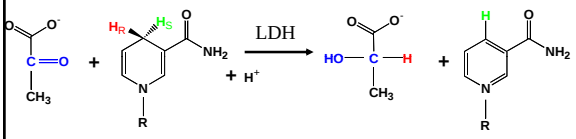


The metabolic fate of pyruvate



The need to regenerate NAD^+ from NADH

A. Homolactic fermentation: conversion of pyruvate to lactate



Mammals have two different types of enzymes: Isozymes

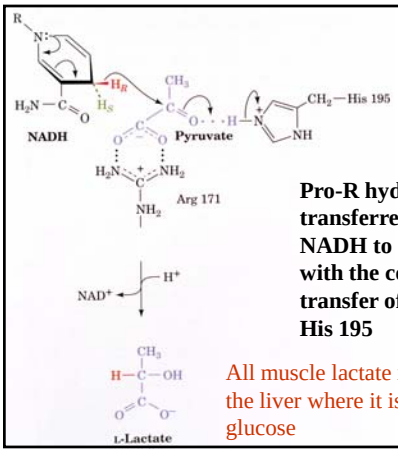
M type for muscle

H type for heart

Lactate dehydrogenase is a tetramer H4 has a low K_m for pyruvate and is allosterically inhibited by high concentrations of pyruvate.

M4 has a higher K_m for pyruvate and is not allosterically regulated

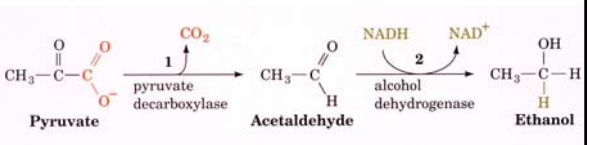
Although all five types can exist, H4, H3M, H2M2 HM3, M4 The M predominates in anaerobic muscle tissues which favor the formation of lactate while the H4 form predominates in aerobic tissues like heart where the formation of pyruvate from lactate is preferred



Pro-R hydride is transferred from C4 of NADH to C2 of pyruvate with the concomitant transfer of a proton from His 195

All muscle lactate is transferred to the liver where it is turned back to glucose

Alcoholic fermentation

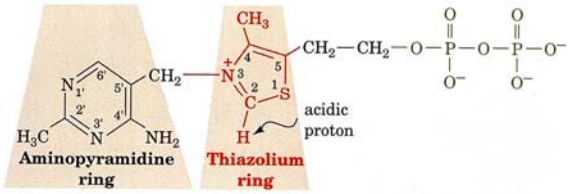


A two step process:

1) Pyruvate decarboxylase requires thiamine pyrophosphate TPP as a cofactor.

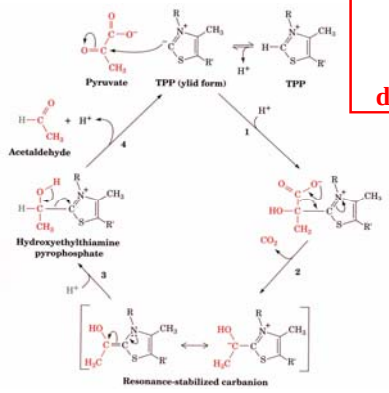
2) Alcohol dehydrogenase requires Zn^{+2} as a cofactor

Thiamine pyrophosphate

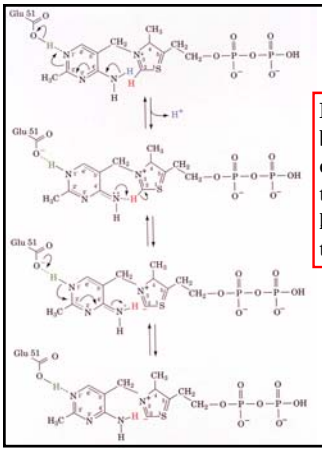


The build up of negative charges seen in decarboxylation reactions on the carbonyl atom in the transition state is unstable and TPP helps stabilize the negative charge

Reaction mechanism of pyruvate decarboxylation



1. Nucleophilic attack by the ylid from of TPP on the carbonyl
2. Departure of CO2 and resonance-stabilization of the carbanion.
3. Protonation of the carbanion
4. Elimination of TPP ylid to form acetaldehyde



Long distance hydrogen bonding and general acid catalysis from Glu 51 with the aminopyrimidine ring leads to the formation of the ylid form of TPP.

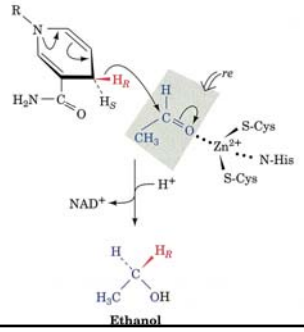
Deficiencies of TPP lead to Beriberi

Vitamin B1

Beriberi was prevalent in the rice consuming countries of the Orient where polished rice is preferred. TPP is found in the brown outer layers of rice.

Neurological atrophy, cardiac failure, endema nowadays found in alcoholics who would rather drink than eat.

Alcohol dehydrogenase



Energetics of Fermentation

	$\Delta G^{\circ\prime}$
Glucose ⟶ 2lactate + $2H^+$	-196 kJ• mol^{-1} of glucose
Glucose ⟶ $2CO_2$ + 2ethanol	-235 kJ• mol^{-1} of glucose

Formation of 2ATP **+61 kJ• mol^{-1} of glucose**

equals 31% and 26% efficient for energy conservation

Under physiological conditions this efficiency approaches 50%

Glycolysis is for rapid ATP production

Glycolysis is about 100 times faster than oxidative-phosphorylation in the mitochondria

Fast twitch muscles - short blasts of energy and are nearly devoid of mitochondria use exclusively glycolysis for ATP

Slow twitch muscles are dark red, rich in mitos obtain ATP from OX-phos., i.e. flight muscles of migratory birds and the muscles of long distance runners

Control of Metabolic Flux

$$S \xrightarrow{J} A \underset{v_r}{\overset{v_j}{\leftrightarrow}} B \xrightarrow{J} P \qquad J = v_f - v_r$$

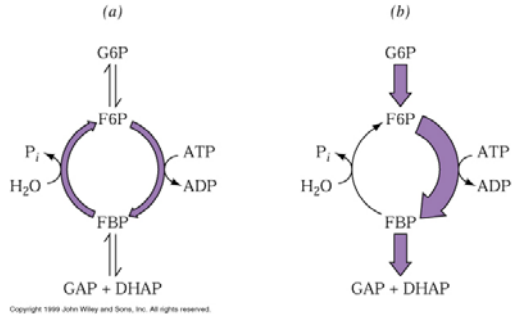
At equilibrium $\Delta J = 0$ and far from equilibrium $\Delta J = v_f$

The flux throughout the pathway is constant at steady state conditions and control of flux requires:

1) The flux-generating step varies with the organisms metabolic needs

2). The change in flux is felt throughout the pathway

A diagrammatic representation of substrate cycling and control of flux

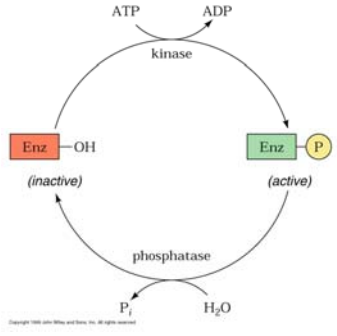


Flux is controlled at the rate limiting step

Usually the product is removed much faster than it is formed so that the rate-determining step is far from equilibrium. Because of the fractional change in the flux $\Delta J/J$ when $v_f >> v_r$ is directly proportional to the change is substrate concentrations other mechanisms are needed to achieve factors of over 100 as seen in glycolysis.

- **Allosteric regulation**
- **Covalent modification**
- **Substrate cycling**
- **Genetic control**

Covalent modification-Protein phosphorylation



Three steps to elucidate common controlling mechanisms in a pathway

1. Identify the rate determining steps: Those with a large negative ΔG and measure flux through the pathway and each step with inhibitors.

2. Identify In vitro allosteric modifiers of the pathway study each enzymes kinetics, mechanisms and inhibition patterns.

3. Measure in vivo levels of modulators under conditions consistent with a proposed control mechanism

Free energy changes in glycolysis

Reaction	enzyme	ΔG°´	ΔG°
1	Hexokinase	-20.9	-27.2
2	PGI	+2.2	-1.4
3	PFK	-17.2	-25.9
4	Aldolase	+22.8	-5.9
5	TIM	+7.9	+4.4
6+7	GAPDH+PGK	-16.7	-1.1
8	PGM	+4.7	-0.6
9	Enolase	-3.2	-2.4
10	PK	-23.3	-13.9

Only three enzymes function with large negative ΔG's

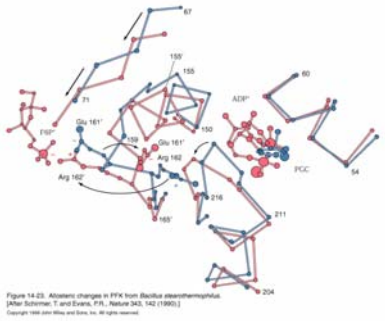
Hexokinase, Phosphofructokinase and pyruvate kinase

The other enzymes operate near equilibrium and their rates are faster than the flux through the pathway.

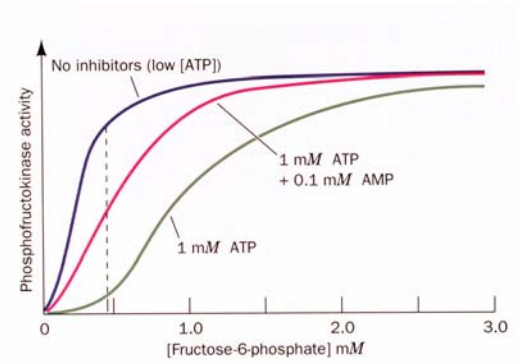
Specific effectors of Glycolysis

Enzymes	Inhibitors	Activators
Hexokinase	**G6P**	**none**
PFK	**ATP, citrate, PEP**	**ADP, AMP, cAMP FBP,F2,6BP, F6P NH_4, Pi**
Pyruvate kinase	**ATP**	**none**

PFK: the major flux controlling enzyme of glycolysis in muscle



PFK activity as a function of G_6P



AMP concentrations not ATP control glycolysis

ATP concentrations only vary about 10% from resting to active cells. [ATP] is buffered by creatine phosphate and adenylate kinase.

$$2ADP \longrightarrow ATP + AMP$$

$$K = \frac{[ATP][AMP]}{[ADP]^2} = 0.44$$

A 10% decrease in ATP produces a four fold increase in AMP because ATP = 50AMP in muscle. AMP activates PFK by the action of adenylate kinase.

Substrate cycling

Fructose-6 phosphate +ATP ⟶ Fructose 1,6-bisphosphate

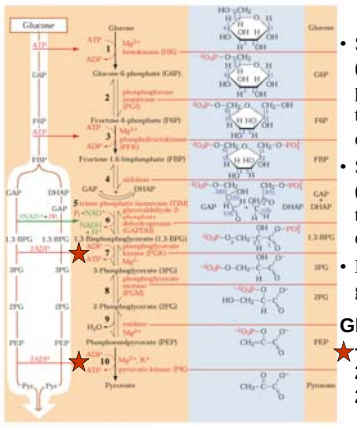
Fructose 1,6-bisphosphate ⟶ Fructose-6 phosphate + Pi

The net result is the breakdown of ATP.

Two different enzymes control this pathway PFK and Fructose 1,6 bisphosphatase. If these both were not controlled a futile cycle would occur.

Specific effectors of Glycolysis

Enzymes	Inhibitors	Activators
Phosphatase	AMP	ATP, citrate



Glycolysis Review

- Stage I: Energy investment (rxns. 1-5), glucose phosphorylated and cleaved to yield 2 G3P and consumes 2 ATP
- State II: Energy recovery (rxns. 6-10), G3P converted to pyruvate with generation of 4 ATP
- Net profit of 2 ATP per glucose

Glucose + 2NAD$^+$ + 2ADP +2P*i* → 2NADH + 2pyruvate + 2ATP + 2H_2O + 4H^+

Next Lecture 24
Thursday 11/12/09
Gluconeogenesis / TCA