Chapter 14
Glycolysis and the catabolism of hexoses

For this chapter you will have to memorize the following for every reaction:
   Name and structure of reactants and products
   Name of enzyme
   Any cofactors required by enzyme
   The $\Delta G^\circ$ of the reaction
   If the reaction is reversible or irreversible
   A good place to start is Figure 14-2 page 545

If the enzyme names are a bit confusing, review table 6-3 page 191 and look at box 16-2 page 646 for an explanation of enzyme names to see how the names fit each reaction.

Glucose under standard state conditions can yield lots of E, -2840kJ/mol by stockpiling glucose as glycogen cell can have a large stockpile of glucose while keeping a relatively low osmotic pressure. Further since glycogen has lots of branch points so it has lots of free ends, the cell can liberate lots of glucose very quickly.

Glucose is not only a fuel, but a basic feedstock for making other compounds. An E coli can use glucose to synthesize every amino acid, nucleotide and fatty acid it requires.

4 major fates of glucose in the cell of a higher plant or animal
   1. Stored (as glycogen, starch, or sucrose)
   2. Oxidized to 3-C compound pyruvate via glycolysis
   3. Oxidized to pentoses via pentose phosphate pathway
   4. Synthesis of structural polysaccharides in the extracellular matrix and cell wall.
This chapter focuses on #2, the reverse of #2 and a bit on #3

14.1 Glycolysis
 glycolysis - sweet splitting
splitting of glucose into 2 3-C units
some of the E is conserved by synthesis of ATP and NADH
Best described and understood metabolic pathway, has been studied since 1890's

Fermentation - general term for anaerobic degradation of glucose to get E in the form of ATP. Glycolysis and fermentation are essentially identical. The only different is the fate of the final product. Under aerobic conditions the 3C product of glycolysis can be further oxidized to yield much more energy but that is the next chapter.

Since early atmosphere didn't contain $O_2$ so oxidation could not occur. This is
probably the most primitive biological mech for getting E from sugars
Yet this pathway is strongly conserved. Enzyme structures are essentially the
same between you and yeast or spinach. The only differences are in the fine
tuning of controls

A. Overview - 2 phases of glycolysis

Figure 14-2
Total of 10 reactions
first 5 are preparatory, breaking glucose into 3C units
Cost 2 ATP to phosphorylate the sugar in the process
last 5 are energy yielding
  1 NADH and 2 ATP are formed from each 3C unit
thus overall cost is
  -2ATP +2 NADH + 4 ATP
For a net of 2NADH and 2 ATP/1glucose
depending on organism and conditions there are 3 fates for the pyruvate
at the end of glycolysis
  1. In aerobic organisms under aerobic conditions
     Pyruvate (CH\textsubscript{3}COCOOH) oxidized to acetate, acetate further
     oxidized to CO\textsubscript{2} in citric acid cycle Chapter 16 in
     mitochondria to generate NADH and FADH used to pump
     proton, out of mito, then protons allowed back in to generate
     ATP (chapter 19)
  2. In aerobic organism under anaerobic conditions (like in muscle
     when you haven’t caught your breath)
     Run out of NADH. Can’t stop ATP synthesis just because
     ran out of NADH, so use NADH to turn pyruvate to
     (CH\textsubscript{3}COCOOH) to lactic acid (CH\textsubscript{3}CHOHCOOH) lower net E
     yield, but allows process to continue. That is why you build
     up lactic acid in muscle
     This process is also done in certain tissues, (brain, retina,
     erythrocytes)
  3. In certain plant tissues, invertebrates, protist under anaerobic
     conditions
     Turn in ethanol (fermentation)

Overall E
Net reaction then is:
Glucose + 2NAD\textsuperscript{+} + 2ADP + 2P\textsubscript{i} →
  2 pyruvate + 2 NADH + 2H\textsuperscript{+} + 2ATP + 2H\textsubscript{2}O
Can separate into two processes

**Exergonic**

\[
\text{Glucose} + 2\text{NAD}^+ \rightarrow 2 \text{pyruvate} + 2 \text{NADH} + 2\text{H}^+ + \Delta G = -146 \text{kJ/mol}
\]

**Endergonic**

\[
2\text{ADP} + 2\text{P}_i \rightarrow 2\text{ATP} + 2\text{H}_2\text{O} \\
\Delta G = +61 \text{kJ/mol (2 x 30.5)}
\]

Net \(\Delta G = -85 \text{kJ/mol}\)

So will go forward

And is so strongly pulled forward that is essentially irreversible

Is only about 40% efficient (61/146)

**Total E**

At this point have recovered a fraction of total E. Can get lots more from total oxidation of pyruvate to CO\(_2\) (Chapter 16 & 19)

**Importance of phosphorylated intermediates**

All 9 glycolytic intermediates are phosphorylated - this has important implications:

1. No transporters for phosphorylated intermediates, so cannot leave cell.
2. Watch as chemistry around phosphate changes energy of linkage until high enough energy to make ATP
3. Binding energy of phosphate group to active site on enzyme lowers activation energy of the respective reactions. Also Mg\(^{2+}\) is usually required to bind ATP, ADP, and many of these phosphorylated intermediates so you will see as a cofactor in many of the enzymes
B. Preparatory reactions (the first 6)

1. Phosphorylation of glucose

\[
\begin{array}{c}
\text{HO-CH}_2 \\
\text{H-OH} \\
\text{O-H} \\
\text{H} \\
\text{OH}
\end{array}
\quad + \quad
\begin{array}{c}
\text{ATP}
\end{array}
\quad \xrightarrow{\text{Mg}^{2+}}
\quad
\begin{array}{c}
\text{HO} \\
\text{O-P-O-CH}_2 \\
\text{H-OH} \\
\text{H} \\
\text{OH}
\end{array}
\quad + \quad
\begin{array}{c}
\text{ADP}
\end{array}
\]

Glucose \quad \text{Glucose 6-phosphate}

\[ \Delta G^\circ = -16.7 \text{ kJ/mole} \]
this is big E drop so irreversible
catalyzed by enzyme hexokinase
(Kinase Enzyme that transfers a \text{PO}_4^- \text{ from NTP to acceptor molecule})
Called hexokinase because will also work with fructose and mannose
absolute requirement for \text{Mg}^{2+} \text{ for binding of ATP-Mg}^{2+}
Back in chapter 8 used this as an example of induced fit because big change in structure when substrate binds
is a soluble cytosolic protein (although may be part of a complex)
Will see later that a step that is both irreversible and initial commit step is a great place for regulation
Hexokinase is present in nearly all organisms
Humans have 4 different hexokinases from 4 different genes (I, II, III, IV)
Different enzymes that perform same reaction are called isozymes
Often involved in different kinds of control in different tissues
2. Conversion of Glu-6-P to Fru-6-P

Enzyme: phosphohexose isomerase (phosphoglucone isomerase)
(Isomerase transfers groups within a molecule to change isomeric form)
Mechanism Figure 14-5
\[ \Delta G^\circ = 1.7 \] so near equilibrium, and reversible
requires \( \text{Mg}^{2+} \)

3. Phosphorylation of F-6-P to F 1,6-bisP

Enzyme : phosphofructokinase -1 (PFK-1)
(Kinase again, \( \text{PO}_4^- \) from ATP to acceptor)
\[ \Delta G^\circ = -14.2 \text{ kJ} \] so strongly favored and irreversible
There is a phosphofructokinase -2
Won’t see until chapter 15 - just mentioning now

PFK-1 again a major E drop and major commit point so is under heavy regulation
perhaps the most complex regulation known
Activity ↑ if ADP or AMP are in excess
Inhibited if excess ATP
More later
Also note naming convention:
- **bisphospho** - means 2 phosphates attached at different places
- **diphosphate** - means a 2-phosphate group attached at a single place (like ADP)
- **trisphosphate** means 3 phosphates at different positions (1,4,5-trisphosphate)
- **triphosphate** means 3 phosphates attached at one place (ATP)

4. Cleavage of fructose 1,6-Bisphosphate

![Chemical structure of fructose 1,6-bisphosphate and glyceraldehyde 3-phosphate](image)

**Fructose 1,6-bisphosphate** ➔ **Glyceraldehyde 3-Phosphate**

Enzyme: aldolase
(Trivial name, not systematic reverse of an aldol condensation?)

ΔG° = 23.8 kJ/mol highly unfavorable but still reversible

next 2 steps rapid so little accumulation of product, so keeps going forward

**Mechanism Figure 14-6**
review reaction of amine with aldehyde to make imine

In vertebrates do not need divalent ion, but in many microorganisms need Zn²⁺ because use a different mechanism

See figure 14-7 for numbering of C through this reaction

5. Interconversion of trioses

![Chemical structure of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate](image)

**Dihydroxyacetone phosphate** ➔ **Glyceraldehyde 3-phosphate**

Enzyme: Triose phosphate isomerase
(Isomerase, internal transfer of a group to change isomer)

ΔG° = 7.5 kJ so not terribly favorable

See figure 14-7
C1 now = C6
C2 = C5
C3 = C4

Finished prep of glucose, now ready to start making E

C. The Payoff phase

6. Oxidation of glyceraldehyde 3-phosphate to 1,3-Bisphosphoglycerate

Enzyme: glyceraldehyde 3-phosphate dehydrogenase
(Dehydrogenase trivial name for an oxidoreductase that removes hydrogen)
$\Delta G^{\circ} = 6.3 \text{ kJ/mol so slightly unfavored}$
Final product contains mixed anhydride or acyl phosphate
very high E substance

Mech is fairly complicated
See figure 14-8

step 1&2  SH for protein adds across aldehyde
(Just like OH does to make hemiacetal but technically thiohemiacetal)
Substrate covalent attached - covalent catalysis

step 3 - 1 H remove as H$^+$ (a pair of electrons)
Thus removing electrons from substrate, and making an oxidation
The hydride is actually transferred to NAD$^+$

Step 4 NADH now leaves enzyme
Phosphate comes in and nucleophilic attack on C=O

Step 5 collapsed product leaves, and SH regenerated

Cell contains limited amount of NAD$^+$ so will grind to a halt here if not regenerated
(Will see how in a minute)
First enz to use NAD$^+$ so lets look at this cofactor in a bit more detail

NAD$^+$/NADH and/or NADP$^+$/NADPH  From 532-535 of text

Nicotinamide adenine dinucleotide NAD$^+$

figure 13-24 from text

difference between NAD and NADP is extra phosphate on c2

vitamin form niacin (nicotinic acid)  
FYI nicotine

Lack of Niacin causes disease pellagra - diarrhea, dermatitis, dementia seen in rural South in early 1900’s
not a true vitamin, can be synthesized from tryptophan
diet of mostly corn, low in tryp, so missing precursor
interestingly corn is rich in niacin, but it is tied up and not available in digestion
  Unless corn soaked in base solution... Hominy!!

Note: look at structure, what is charge on NAD$^+$ ?? -2+1 = -1
the + in NAD$^+$ indicates only the charge on the base, not that whole molecule, is mostly a reminder that is in oxidized form

so NAD$^+$ NADP$^+$ refers to oxidized forms, NADH and NADPH refers to reduced forms
NAD and NADP generic term if you don’t care if oxidized or reduced

NAD/NADP always involved in 2 electron reactions involving a hydride ion( H:)\textsuperscript{-}
change ins structure shown in figure
In most enzymes reaction is stereospecific, and H will add from one side or the other but not both

Net reaction

NAD(P)$^+$ + 2e$^-$ + 2H$^+$ = NAD(P)H + H$^+$
  (Technically H$^+$ is a spectator but will always see as product in biological reactions)
UV characterizes change when oxidized or reduced. Make it easy to follow reaction if you have a spectrophotometer that goes into UV (as saw in labs last semester).

Total conc. NAD + NADH is about $10^{-5}$ molar
Total conc. NADP/NADPH $10^{-6}$ molar

Since chemically almost identical, reduction potential of NAD and NADP is essential the same, YET

NAD$^+$/NADH couple is used extensively in catabolic metabolism, that is, oxidizing things to get E,
$[\text{NAD}^+]/[\text{NADH}]$ large ($[\text{NAD}^+]$ high) so reaction driven to right,

NADP$^+$/NADPH is used extensively in anabolic metabolism, that is reducing things that were oxidized into new useful compound.
$[\text{NADP}^+]/[\text{NADP}]$ is very low, ($[\text{NADPH}]$ high) so reaction driven to left

Most enzymes will use one form but not the other
this keeps anabolic metabolism separate from catabolic so don’t get futile cycles

Association between NAD and enzyme is very loose, so in most mechanisms NAD is bound in one step and then released in another. NAD is a soluble way to move electrons around in the aqueous cytosol of a cell.
Returning to metabolism

7. Phosphoryl transfer from 1,3-Bisphosphoglycerate to ADP

Enzyme: phosphoglycerate kinase
\[ \Delta G^0 = -18.5 \text{ kJ} \] big E drop

note name kinase, name actually refers to reverse reaction! Does the reverse reaction in photosynthesis but not in glycolysis

Large E drop here used to pull previous reaction or three along

Glyceraldehyde 3-phosphate + NAD\(^+\) ⇌ 1,3-bisphosphoglycerate + NADH + H\(^+\)  
1,3-bisphosphoglycerate + ADP ⇌ 3-Phosphoglycerate + ATP

NET:
Glyceraldehyde 3-phosphate + NAD\(^+\) + ADP ⇌ 3-Phosphoglycerate + ATP + NADH + H\(^+\)

-12.2

Plus some E left over to pull even more this way

Synthesis of ATP by direct transfer of a phosphate group from the substrate called

**substrate-level phosphorylation**

as opposed to *respiration-linked phosphorylation* that we will see later in respiration
8. Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

![Chemical structures of 3-Phosphoglycerate and 2-Phosphoglycerate](image)

Enzyme: phosphoglycerate mutase  
(mutase - trivial name for an isomerase?)  
$\Delta G^\circ = 4.4 \text{ kJ} \ \text{reversible}$

Enzyme has an interesting mechanism. Instead of simply moving the phosphate from one OH to the other what actually happens is this:

**Figure 14-9**

The enzyme contains a Phosphorylated His  
this phosphate is attached to make 2,3- biphosphoglycerate  
the original phosphate at the 3 position is then left on the enzyme at the his  
so the enzyme is ready for the next cycle

3 additional points

1. How does get phosphorylated to begin with?  
   3 phosphoglycerate is phosphorylate from ATP via a kinase to make 2,3-Bis  
   That then acts like a co enzyme

2. In most cells 2,3-biphosphoglycerate is only in trace amounts in RBC is at 5 mM Do you remember why?  
   (Part of regulation of hemoglobin and $O_2$ binding. Used for altitude adaption)

3. Will see other enzymes with same mechanism
9. Dehydration of 2-phosphoglycerate to phosphoenolpyruvate

\[
\begin{align*}
\text{2-Phosphoglycerate} & \quad \text{Phosphoenolpyruvate} \\
\end{align*}
\]

Enzyme: Enolase  
(Another trivial name)  
\[\Delta G^\circ = 7.5 \text{ kJ/mol}\]

Small change in \(E\) of product vs reactant  
but big change in \(E\) of breaking phosphate  
hydrolysis of phosphate in 2-Phosphoglycerate would yield \(-17.6\) kJ  
hydrolysis of phosphate in PEP would yield \(-61.9\) kJ of \(E\)!  
So removal of water has greatly increased the potential \(E\) we can get from  
this phosphate  
Can you see why?  (Organic chemists should recognize that OH next to a  
double bond is not a favorable linkage)

10. Transfer of phosphorous group for PEP to ADP

\[
\begin{align*}
\text{Phosphoenolpyruvate} & \quad + \quad \text{ADP} & \rightarrow & \quad \text{Pyruvate} & \quad + \quad \text{ATP} \\
\end{align*}
\]

Enzyme: pyruvate kinase  
(Again kinase is the reverse reaction)  
\[\Delta G^\circ = -31.4\]

essentially irreversible  
make it a good control point  
another substrate level phosphorylation  
another enzyme named for the reverse reaction!  
Large amount of the energy comes from the fact that in  
phosphoenolpyruvate you are locked into a very unfavorable enol form.
Molecule very much prefers to shift to a keto form
(See figure left column page 555)

D. Overall balance sheet
Glucose + 2NAD$^+$ + 2 ATP + 4ADP + 2P$_i$ $\rightarrow$
2 pyruvate + 2 NADH + 2H$^+$ + 2ADP + 4 ATP + 2H$_2$O

for a net of
Glucose + 2NAD$^+$ + 2ADP + 2P$_i$ $\rightarrow$
2 pyruvate + 2 NADH + 2H$^+$ + 2ATP + 2H$_2$O

Under aerobic conditions the 2 NADH are transferred to the mitochondria
where the can be changed back to NAD$^+$ and, in the process generates
additional ATP via respiration linked phosphorylation.
Essentially: 2NADH + 2H$^+$ +O$_2$ + 2.5 ADP + 2.5 Pi $\rightarrow$ 2NAD$^+$ + 2H$_2$O + 2.5
ATP (or 1.5 ATP if use alternate shuttle)

Intermediates are channeled between glycolytic enzymes
All of the above enzymes usually described at soluble components of the
cytosol

presently suspect that this may be an artifact of purification process

when at realistic concentrations, spontaneously form high level
aggregates held together via non-covalent interactions

Complexes much more efficient because allow Substrate to channel (go
directly from one enzyme to the next) without going into the bulk solution

Lots of evidence to support, but no detailed model a this time

E. Glycolysis is under tight regulation
‘Pasteur effect’ rate and total amount glucose used is much higher under
anaerobic conditions than aerobic.
Essentially need about 15x more glucose for same amount of ATP
because not as efficient as aerobic metabolism.
Cell tries to keep ATP levels constant
So interplay between ATP consumption, NADH regeneration and
other factors to keep cell in proper balance. Will study details in
next chapter.
For now will focus on 2 medical implications:

1. Glucose uptake and glycolysis about 10X faster in solid tumors than in normal tissue
   Often outstrip their O₂ supply because usually not many capillaries
   So must rely on glycolysis for E
   Typically cancerous cells are low on mitochondria and overproduce the glycolytic enzymes
   See Box 14-1 page 556-557 for more details

2. Type 1 Diabetes Mellitus (figure 14-10)
   Back in Chapter 11 discussed Glucose transport. There was a whole family of glucose transporters GLUT 1-GLUT 12. GLUT 4 is the main transporter in skeletal muscle, cardiac muscle and adipose tissue. It is sequestered in intracellular vesicles that only fuse with plasma membrane in response to insulin signal, when insulin is released from the pancreatic β cells in response to elevated blood glucose. In Type 1 diabetes, you have too few β cells, so you don’t make enough insulin to get themuscles and adipose tissue to transport glucose out of the blood. Two effects:
   1. You have high sugar levels in blood (hyperglycemia)
   2. Muscle cells don’t have enough energy so start breaking down fats (triacylglycerols). To help this along the adipose tissues start breaking down fats into acetyl CoA. In the liver this acetyl CoA is converted into acetoacetate and β-hydroxybutyrate, the commonly called ‘ketone bodies’ that are then used in the muscle as an energy source. It is these ketone bodies that are acidic and lower the pH of the blood causing ketoacidosis
14.2 Feeder Pathways for Glycolysis

A. Dietary Polysaccharides and Disaccharides hydrolysed to Monosaccharides

Starch
digestion begins with \( \alpha \)-amylase in saliva hydrolyzes the \( \alpha 1-4 \) linkages in starch to make short oligo- and polysaccharides

\( \alpha \)-amylase inactivated by low pH of stomach, but a second form of \( \alpha \)-amylase is secreted by pancreas to continue digestion in small intestine. In the small intestine starch continues to be broken down into 2 and 3 sugar units (maltose and maltotriose) and the limit dextrins with the 1-6 linkages

These 2 and 3 unit sugars are degraded into glucose by then enzymes in the intestinal brush border, and the glucose is transferred into the blood as discussed in chapter 11

So Starch enters as Glucose

Glycogen
Since its structure is essentially the same as starch, it is metabolized the same way.

Cellulose
most animals lack the cellulase necessary to break the \( \beta 1-4 \) linkage ruminants have an extended stomach where microorganisms can break down the cellulose, then perform anaerobic fermentation on the glucose to take it to propionate. The propionate is then used in gluconeogenesis to form lactose.

B. Endogenous glycogen and starch are degraded by phosphorolysis
Glycogen in your liver or muscle is broken down by a different mechanism Glycogen phosphorylase or similar enzyme in plants (Figure 14-12) inorganic phosphate used to cleave \( \alpha 1-4 \) linkages

Yields Glu -1-P
Need phosphoglucomutase to move P to the 6 position to start glycolysis
This enzyme needs a 1,6, biphosphoglucone much like our earlier phosphoglyceratemutase needed a 1,3-bisphosphate glycerate, only in this case the \( PO_4 \) is linked to a ser on the enzyme instead of a His
Called phosphorolysis because uses phosphate to split linkage Requires cofactor called pyridoxal phosphate
will work until 4 away from a branch point (see figure 15-28)
debranching enzyme then does 2 things

1. Moved three end sugars to non-branched end
2. Removes 1-6 linkage to release glucose (no P)

So Glycogen enters primarily as glucose -1-P

Examine figure 14-11 for other monosaccharides, otherwise skip other details

Dissacharides must be hydrolyzed to monosaccharides before entering the cell. This is done by enzymes attached to the outer surface of the intestinal epithelial cells.

Lactose intolerance come from the disappearance of lactase activity from the intestinal epithelial. When the undigested lactose hit the large intestine bacteria convert it to toxic product that cause cramps and its presence increases the osmolality So more water is retained.

C. Other monosaccharides enter the glycolytic pathway at several points
The book spends another page discussing how some of the other monosaccharides shown in Figure 14-11 get into glycolysis. I may discuss quickly in class, but let’s not worry about the details

14.3 Fates of Pyruvate under Aerobic and Anaerobic conditions
Under ideal aerobic conditions, NADH and pyruvate can both be shuttled into the mitochondria where NADH is converted back to NAD⁺ to generate E₂, and the pyruvate is completely oxidized to CO₂
NADH shuttle is indirect, through Malate/Aspartate shunt Chapter 19

Under anaerobic conditions need to regenerate NAD⁺ or everything grinds to a halt
In animal tissues this is usually done by reducing pyruvate to lactate
(See figure right column page 563)
enzyme: lactate dehydrogenase (again named for reverse reaction)
ΔG°° = -25.1 kJ large and favorable

This build up lactic acid ‘burn’ for athletes
lowers pH causes pain and limits amount of activity
excess lactate put into blood, goes to liver, regenerated to Glucose then to glycogen
Also done by lactobacilli and streptococci
When happens in milk, as pH drops proteins ppt out and you get cheese
and yogurt
in sausages get the ‘tang' of a summer sausage

B. Ethanol is the reduced product in Ethanol Fermentation
In yeast and other microorganisms
See reaction left column  565
decarboxylate, (pyruvate decarboxylase) release CO$_2$
Reduce to ethanol (alcohol dehydrogenase)

Used to make alcoholic beverages, or CO$_2$ for bread to rise
same enzyme alcohol dehydrogenase then used by your body to
metabolize ethanol to acetaldehyde so it can be further metabolized

C. Thiamine pyrophosphate carries “Acive Acetaldehyde” groups
Skip Thiamine pyrophosphate (much as I hate to)

D. Fermentations in Common food and Industrial Chemicals
skip microbial fermentations

14.4 Gluconeogenesis
Most organisms synthesize glucose from simple precursors like pyruvate or
lactate.  Process called Gluconeogenesis

In mammals occur primally in liver to make glucose for export to other tissues
Your brain alone need 120g a day of glucose (maybe more during finals?)

Uses many of the same enzymes, but is not simply reverse
(Cannot be, for that would be energetically unfavorable)

The 7 reversible reaction are: 2,4,5,6,7,8,9 so same enzyme used in both
directions

The three nonreversible reactions are 1,3, and 10 (hexokinase, PFK-1 and
pyruvate kinase (figure 14-17)

In these cases uses an alternative reaction so is irreversible in opposite direction

Let’s look at the details of this reverse process, and then, in the next chapter we
will discuss how the two processes (glycolysis and gluconeogenesis) are
controlled.
A. Conversion of pyruvate to phosphoenolpyruvate requires 2 reactions
   In glycolysis PEP to pyruvate \( \Delta G = -31.5 \) kJ so a big E drop to reverse
I. Pathway 1 used then pyruvate or alanine are source
   Step 1 - Move pyruvate into mitocondria
      (Or make pyruvate from alanine in mitochondria Chapter 18)
   Step 2 Pyruvate carboxylase (fig 14-18)
      Product is oxaloacetate
      Requires ATP E to push along
      Requires biotin (figure 14-19)
      Acts as activator and carrier of \( \text{CO}_2 \)
      Regulatory step
   Step 3 Move oxaloacetate back into cytosol where rest of reactions occur
      No transporter for oxaloacetate in mito!!
      Have to hydrogenate to malate
      Then in cytosol dehydrogenate back to oxaloacetate
      (Show structures on board)
      Will see this trick again in chapter 16 to get NADH equivalents out of mitochondria
      Net is transfer of NADH equivalent from mitochondria to cytosol
   Step 4 PEP carboxykinase
      Use GTP to push along

Net
Pyruvate + ATP + GTP + HCO_3^- \rightarrow PEP + ADP + GDP + Pi + \text{CO}_2
Std free E 0.9 kJ/mol so would think near equilibrium
Free E in cell actually about -25 kJ so strongly favorable
II. Pathway 2 used when lactate dominates

Red blood cells, and muscle without O₂ (lactate generation)

Figure 14-20

Glycolysis: Glucose + 2 NAD⁻→pyruvate + 2NADH

Pyruvate + 2NADH → lactate + 2NAD

So no net change NAD/NADH

If you have lactate build up, reverse this last reaction to remake pyruvate

Step 1. Pyruvate to mitochondria

(Same as other pathway)

Step 2. Pyruvate to oxaloacetate

(Same as other pathway)

Step 3 Oxaloacetate to PEP

Using mitochondrial PEP carbocyclase

Step 4 transport PEP to cytosol

B. Fructose 1,6-Bisphosphate to Fructose 6-Phosphate

fructose 1,6-bisphosphatase (FBPase-1)

Mg²⁺ dependent

essentially irreversible (std E = -16.6 kJ)

Simply remove the phosphate

C. Glucose 6-Phosphate to Glucose

glucose 6-phosphatase

Mg²⁺ dependent

again essentially irreversible (std E -13.8 kJ)

Lets look at how these three enzymes are regulated

Enzyme missing in brain and muscle, so these tissues cannot make glucose

Only source of glucose is from blood

D. Gluconeogenesis is expensive

2 pyruvate + 4 ATP + 2 GTP + 2NADH + 2H⁺ +4H₂O →

Glucose + 4ADP + 2 GDP + 6Pi + 2NAD⁺

Std E ~ - 16 kJ

clearly not the reverse of glycolysis

Glucose + 2 ADP + 2 Pi + 2 NAD⁺→ 2 pyruvate + 2 ATP + 2NADH + 2 H⁺ + 2 H₂O

actual E ~ -63 kJ

Skip to 14.5 Pentose Phosphate pathway

14.5 Pentose Phosphate

Major fate of glucose is glycolysis, but there is another fate, the pentose phosphate pathways
also called the phophogluconate pathway

2 reasons to use this way

1. To make NADPH
   - Have seen and will see NADH as high E intermediate used to send reducing equivalents to oxidation phosphorylation
   - NADPH is similar in structure, but contains an extra phosphate
   - Used to put reducing equivalents into synthetic pathways
   - Needed in cells synthesizing fatty acids or steroids
     - (Mammary gland, adrenal cortex, liver, adipose tissue)

2. To make 5C sugars (ribose for RNA and DNA)
   - Will see in growing tissue and tumors

Won’t study details of this pathway, just want to give you a feel for it

Phase 1 oxidation of Glu-6-P to Ribose-5-P **Figure 14-22**
- generates 2 NADPH

Phase 2 non-oxidative shuffle of three 5C sugars to 2 6C sugars and glyceraldehyde 3-p
- (Needed in tissues that are generating NADPH, because phase 1 would generate more 5C sugars than the cell needs, so here we get back to 6C sugars)
  - **Figure 14-23**
  - this part readily reversible

Also used by plants for CO₂ fixation

**Gluconeogenesis Net:**
- 2 pyruvate + 4 ATP + 2 GTP + 2NADH + 2H⁺ + 4H₂O →
- Glucose + 4ADP + 2GDP + 6 Pi + 2NAD⁺

**Glycolysis Net**
- Glucose + 2 ADP + 2Pᵢ + 2NAD⁺ →
- 2 Pyruvate + 2 ATP +2NADH + 2 H₂O

So if simply cycle would be very wasteful
Next chapter about how to keep separate from each other, in both occur in cytosol and use many of the same enzymes!