Chapter 13: Carbohydrate Metabolism I

The first sequence of biochemical reactions we will be discussing is glycolysis. A summary of the glycolytic pathway is described very well on p. 458 of Mathews. Glycolysis is the catabolism of glucose to two molecules of pyruvate. This sequence of reactions results in the net synthesis of two molecules of ATP and two molecules of NADH, which have the capability of creating ATP through the electron transport chain. As we talk about glycolysis, we will determine which reactions are energetically unfavorable and how these reactions are allowed to occur. We will also examine some of the mechanisms of reactions that are part of glycolysis.

The first step in metabolism is the transport of glucose into the cell, which we recently discussed. In the liver and erythrocyte (red blood cell), glucose is transported into (and out of) the cell via a carrier mediated passive transport system. Let’s take some isolated erythrocyte membranes, and control membranes, and suspend them between two compartments. D-glucose is added to one compartment, and the rate of movement of the sugar into the second compartment through the membrane is measured. Here are the results:

First, let’s look at the control situation, where there is a synthetic lipid bilayer separating the two compartments. What do these results tell you? These results tell you that there is no glucose transport between the two compartments if a lipid bilayer is in place. This is expected, since glucose is a very polar molecule, and you remember from last semester that polar molecules cannot pass through bilayers very easily.

Now let’s look at the erythrocyte membrane. What do these results tell you? These results tell you that the glucose concentration on the two sides of the compartment come to equilibrium at the same concentration. This suggests that there is something (protein) in the erythrocyte membrane that facilitates the transport of glucose from one side to the other. It also tells you that energy is not required, and that glucose transport is bidirectional, depending upon the relative glucose concentrations on the two sides of the membrane.

Finally, let’s examine glucose transport when brush border epithelial membranes are used. What do these results tell you? These results tell you that in brush border membranes, the extent of glucose transport from one side of the membrane to the other is much greater than expected from nonmediated diffusion, or as measured in control membranes. The conclusion of
these experiments is that there is a protein transporter in the brush border membrane that will allow the transport of glucose across the membrane.

Four different glucose transporters have been identified. These are very similar to each other in amino acid sequence (and presumably structure), but are expressed in different tissues. GLUT1 is expressed in most tissues, but only in tiny amounts in liver and muscle, the glucose hogs of our bodies. GLUT2 is prominent in pancreatic β-cells (why is this?) and in liver. GLUT4 occurs mainly in muscle and fat cells. We’ll return to glucose transporters later in the semester when we discuss metabolic control.

Getting back to glycolysis, and concentrating on the liver, the liver transporter is an equilibrium transporter. If we relied only on the transporter, only one-half of the glucose exposed to a cell would be absorbed. This is not a very efficient way of doing things. In order to optimize transport, the glucose which is transported into the cell is immediately converted into another molecule. Once another molecule, there is less glucose inside the cell, and more can be transported across the membrane. The cell traps glucose in the cell by adding a phosphate group to the 6-hydroxyl group by the action of the enzyme hexokinase. Hexokinase catalyzes the phosphorylation of glucose, with phosphate donated from ATP.

This modification of glucose successfully traps glucose in the cell by two mechanisms: 1. It makes glucose highly charged, so it has more difficulty in traveling through the cell membrane, and 2. It reduces the cellular glucose concentration, decreasing the glucose concentration gradient across the cell membrane.

Note from Table 13.1 that the standard free energy change for this reaction is -16.7 kJ/mol, while the actual free energy change (taking cellular concentrations into account) is -33.5 kJ/mol. What does this tell you about the relative concentrations of ATP, glucose, and glucose-6-phosphate in a cell?

The chemical reaction occurring is a phosphoryl transfer reaction, a very common biochemical reaction. An enzyme which catalyzes a phosphoryl transfer reaction is given the general name of kinase. I want to take a moment to attempt to talk about how the energy released in the hydrolysis of a phosphoanhydride bond is used to drive the phosphorylation of a carbohydrate by inorganic phosphate. These reactions are carried out by enzymes, and in most cases the first step of the enzymatic reaction is the binding of ATP to the enzyme. This
statement isn't exactly true; for almost all enzymes which use ATP as a substrate, the true substrate is not ATP at all, but ATP with a Mg ion bound to the phosphate oxygens. (SEE OVERHEAD)

The Mg will chelate to two negatively charged oxygen atoms on the phosphates. In addition, an arg side chain is commonly found near the terminal phosphate; the positive charged on the guanidino group will coordinate with the electronegative oxygens on the terminal phosphate. Another common constituent of the active site is a lysine which will coordinate with the first phosphate. By the way, the phosphate groups on ATP are designated alpha, beta, and gamma, in their order beginning at the phosphate attached to the ribose sugar ring.
By putting all of these positive charges around the phosphate oxygens, electron density is pulled away from the phosphate, making it somewhat electrophilic. A nucleophilic second substrate will bind and attack the phosphate, forming a covalent bond with the phosphate while at the same time breaking the P-O bond between the gamma and beta phosphate groups. This process is facilitated by the enzyme, which properly aligns the second substrate to best attack the terminal phosphate.

There are many enzymes which carry out this reaction, as we will see throughout the semester. Some, like hexokinase, are used to convert ATP into ADP and a lower energy organic phosphate. Those organic phosphates which have lower energy than ATP, such as glucose-6-phosphate, will yield the organic phosphate + ADP when reacted with the carbohydrate and ATP. However, if the organic phosphate has higher energy than ATP, the favored reaction will be the reverse reaction, that is, the synthesis of ATP from the organic phosphate and ADP. An example of such a reaction is catalyzed by the last enzyme in glycolysis, pyruvate kinase, and involves the transfer of a phosphate group from phospho(enol)pyruvate to ADP to form ATP. The mechanism of the enzyme and the steps are the same. The direction of the reaction is almost exclusively controlled by the relative energies of reactants and products. The reaction will occur in the direction which gives off the most energy; which generates the products of the lowest energy.

The next glycolytic reaction that I would like to discuss in detail involves the breakdown of a 6-carbon compound into two 3-carbon compounds. It is catalyzed by the enzyme aldolase, and converts fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate:

The reaction is the reverse of the aldol condensation in organic chemistry- a diol is converted into an alcohol plus an aldehyde.

Referring to the Table, the standard free energy change for the aldolase reaction appears to be very positive (+23.9 kJ/mol), such that the reaction would not occur in vivo as written. However, the fact that two different reaction products are formed from a single substrate provides instant help in pushing the reaction forward. If you remember the expression for determining \( \Delta G \):

\[
\Delta G = \Delta G^0 + RT \ln \left( \frac{[\text{products}]}{[\text{reactants}]} \right)
\]
In this case, two molecules of product are formed for each molecule of substrate. If the product concentration is somewhat less than the substrate concentration, the equilibrium expression magnifies this difference, and the energy barrier for the reaction is greatly decreased.

The mechanism of the enzyme aldolase requires the formation of a covalent intermediate. The first step of the reaction requires the formation of a Schiff's base with the C-2 ketone function on fructose-1,6-bisphosphate: (SEE power point slide). Water is lost upon formation of the Schiff's base. The nitrogen in the Schiff's base is protonated, and glyceraldehyde-3-phosphate is released from the enzyme, leaving the enolate anion covalently bound to aldolase:

The proton on the Schiff's base transfers to the carbanion, then the Schiff's base is hydrolyzed by water to form the final product. Note that this mechanism of aldolase defines how the products of the reaction are derived from the substrate. Carbons 1-3 on fructose-1,6-bisphosphate go to dihydroxyacetone phosphate, while carbons 4-6 wind up as glyceraldehyde-3-phosphate.

Dihydroxyacetone is not directly on the glycolytic pathway, but is easily converted enzymatically to glyceraldehyde-3-phosphate by the enzyme triose phosphate isomerase. (OVERHEAD) This reaction mechanism associated with the reaction works via a cis-enediol intermediate. An acidic residue (his-95) in the active site of triose phosphate isomerase donates a proton to the carbonyl oxygen of dihydroxyacetone phosphate. At the same time, a basic residue (glu 165) grabs a proton from the alcoholic carbon, and electrons rearrange to form the cis-dienol intermediate. It’s cis because the two oxygens are found on the same side of the double bond; these are stabilized by lys-12 side chain in the active site. The proton on glu 165 is donated to the center carbon, and at the same time the proton is removed from what was originally an alcohol and the electrons rearrange to form the carbonyl group at the terminal carbon. The energetics of the reaction favor the dihydroxyacetone phosphate, but the glyceraldehyde-3-Pi is carried further through glycolysis, and is replenished by the isomerization reaction.

Let’s review the energetics of glycolysis to this point. In converting glucose into effectively two molecules of glyceraldehyde-3-phosphate, the energy of 2 ATP molecules have been invested into glucose without any energy return, one at the hexokinase step and one at the
phosphofructokinase step. The function of glycolysis is to oxidize glucose with concurrent generation of energy. But at this point in glycolysis, the energy harvest begins. The first energy producing step is catalyzed by the enzyme glyceraldehyde-3-phosphate dehydrogenase, which catalyzes the phosphorylation of glyceraldehyde-3-phosphate with the concurrent reduction of NAD$^+$:

Oxidation of the aldehyde requires removal of a hydride ion and transfer to NAD$^+$. This is a difficult task with an aldehyde due to the partial positive charge on the aldehyde carbon as the result of the carbonyl group; removal of a negatively charged hydride ion is very unfavorable. To aide in the removal of the hydride ion, a nucleophile must be added to the aldehyde. In glyceraldehyde-3-phosphate dehydrogenase, the nucleophile is the sulfhydral group on an active site cysteine. After addition of the sulfur group to the aldehyde, the hydride ion is removed relatively easily by the oxidized form of NAD.

Now would be a good time to review the structure of NAD (nicotinamide adenine dinucleotide) and how it functions as an electron transfer molecule. This molecule was introduced in your textbook in Chapter 11 (p. 389) along with enzyme cofactors. NAD consists of a nicotinamide group, connected to a ribose diphosphate, connected in turn to an adenosine nucleoside. NAD is the acceptor molecule for electrons in oxidation reactions. For most biosynthetic reactions that require electrons for reduction reactions, the cofactor that is used is NADP – nicotinamide adenine dinucleotide phosphate – where the ribose sugar on the adenosine nucleoside has a 2’-phosphate group.

The nicotinamide group can exist in either an oxidized form or a reduced form. In catabolism, electrons are accepted by the oxidized form, resulting in the reduced form. The oxidized form of the nicotinamide ring has this structure; the ring has a net positive charge centered on the pyridinium nitrogen. When the ring is reduced, two electrons and a proton are added to the ring (in the form of a hydride ion), generating an aromatic system with no charge. These electrons are donated to a mitochondrial system called the electron transport chain, which leads to ATP synthesis; we’ll talk about this much more in Chapter 14. At this point, all I want to mention is that the energy stored in two electrons is converted by electron transport into three moles of ATP per mole of reduced carrier.

So, addition of the sulfur leads to an intermediate that is susceptible to hydride removal; the removal of the hydride ion by NAD$^+$ results in a thioester intermediate. This is an
energy-rich intermediate. An orthophosphate group is capable of attacking the thioester, resulting in the free enzyme and 1,3-bisphosphoglycerate. So, the coupling mechanism between the aldehyde oxidation and the phosphorylation is the thioester.

Let’s briefly look at the 1,3-bisphosphoglycerate and examine the energetics of the molecule. How would you describe the bond connecting the phosphate group at C1 of the molecule? This is a phosphoanhydride bond, and you can imagine that it has a great deal of energy associated with it. On the other hand, the phosphate group on C3 is connected via a simple ester linkage, and it does not contain a great deal of energy.

This reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase is important in terms of energy production in two ways. First, a molecule of NADH is formed, which can later be converted to ATP via the electron transport chain and oxidative phosphorylation. (Since 1 molecule of glucose forms 2 molecules of glyceraldehyde-3-phosphate, the net recovery of NADH from this step is 2 NADH/1 glucose). Second, glyceraldehyde-3-phosphate is phosphorylated without the expense of an ATP molecule. The standard free energy of hydrolysis of the phosphate group on the 1 position of glyceraldehyde-1,3-bisphosphate is -11.8 kcal/mol. This is more negative than that for ATP, and this means that an ATP molecule can be formed by a phosphoryl transfer reaction between glyceraldehyde-1,3-bisphosphate and ADP.

This reaction is the next step in glycolysis, and is catalyzed by the enzyme phosphoglycerate kinase. The reaction product is 3-phosphoglycerate. The final steps in glycolysis involve the conversion of 3-phosphoglycerate to pyruvate with transfer of the high energy phosphate group from the organic compound to ATP. The next step in the reaction is a phosphoryl shift from the 3 to the 2 position of phosphoglycerate, catalyzed by the enzyme phosphoglycerate mutase. Note the nomenclature difference between an isomerase and a mutase; a mutase is also an isomerase, but involves the migration of a phosphate group from one alcohol to another. The next step is a dehydration reaction, changing the phosphate ester group of 2-phosphoglycerate into the enol group of phosphoenolpyruvate. The reaction is catalyzed by the enzyme enolase. The enol group has a much higher standard free energy of hydrolysis associated with the phosphate group than the phosphate ester. The ester has a free energy of hydrolysis of -15.6 kJ/mole, while the phosphate group of phosphoenolpyruvate has an associated free energy of hydrolysis of -61.9 kJ/mol, more than sufficient to power the
phosphoryl transfer to ATP.

The final step in glycolysis is this phosphoryl transfer reaction, catalyzed by the enzyme pyruvate kinase. The phosphate group on the phosphoenolpyruvate is transferred to ADP, synthesizing ATP.

Let’s summarize the energy gain from glycolysis. For each glucose molecule:

-1 ATP   phosphorylation of glucose
-1 ATP   phosphorylation of F-6-P
+2 NADH  phosphorylation of glyceraldehyde-3-P
+2 ATP   dephosphorylation of glyceraldehyde-1,3-bisP
+2 ATP   dephosphorylation of PEP

We’ll be discussing erythrocytes later in this class period; let me take a minute to look at the bioenergetics of this specialized cell. Who can tell me something about the cell biology of erythrocytes? (denucleated, no mitochondria). If the NADH go to the mitochondria for energy generation, if there are no mitochondria, what happens to these? They are used to synthesize NADPH, which is used for reduction reactions beneficial to the organism; we’ll look at this again when we visit the pentose phosphate pathway in a few chapters. Since there is no mitochondria, energy metabolism in erythrocytes ends at pyruvate. Only 2 ATP are generated for each molecule of glucose consumed. This is actually OK, since the erythrocyte has very little energy needs. It is essentially a bag of hemoglobin, so it needs very little energy. Most of the energy needed by erythrocytes is needed to drive its Na+/K+ ATPase to maintain ion balance in the cell; about half of its energy drives this transport system.

FERMENTATION

I would like to take a few minutes to discuss the different things which might happen to pyruvate after its formation. Under normal circumstances, pyruvate is transported into the mitochondria and further oxidized to carbon dioxide and water, with the generation of chemical energy. We’ll discuss this process in a few weeks when we discuss the citric acid cycle and oxidative phosphorylation. However, depending upon the type of organism and its environmental state, pyruvate might be used for other reactions.

In mammalian cells, under conditions where the cell is deprived of oxygen (which is required for oxidative phosphorylation), or if the cell is lacking in mitochondria, pyruvate
concentrations may build up high enough to allow for the action of the enzyme lactate dehydrogenase. Lactate dehydrogenase catalyzes the reduction of pyruvate to lactate, with corresponding oxidation of NADH to NAD+.
Lactate is a stable end product of glucose metabolism, and can be excreted into the bloodstream for elimination. (Pyruvate is unable to pass through the plasma membrane of cells). If we return to our energy balance, we find that for the production of lactate from glucose, 2 molecules of ATP are utilized, 2 molecules of NADH and 4 molecules of ATP are generated, and finally the 2 molecules of NADH are oxidized. Therefore, the net energy gain by the cell from the metabolism of glucose to lactate is 2 molecules of ATP. Since over 30 molecules of ATP are generated from glucose through oxidative phosphorylation, lactate production is not a very efficient way for a cell to use glucose. However, in cases where oxygen is deprived, lactate production allows a cell to derive a little energy from glucose. The production of lactate from pyruvate is necessary in order to regenerate NAD+ for use by glyceraldehyde-3-phosphate dehydrogenase. If the NADH was allowed to accumulate, the result would be product inhibition of this enzyme and shutdown of glycolysis.

Your textbook points out that even in normally functioning muscle cells, the conversion of glucose to lactate may represent a significant amount of the glucose metabolism in muscle. Muscle can be divided into fast twitch and slow twitch muscle. Who knows the anatomical difference between fast and slow twitch muscle? (Dark meat and white meat). Dark meat is dark because it is rich with mitochondria. Mitochondria contains many cytochrome proteins; these proteins contain iron and are red. Slow twitch muscle have the ability to convert a great deal of pyruvate into carbon dioxide. However, fast twitch muscle are deficient in mitochondria, and have little ability to convert pyruvate into carbon dioxide. Fast twitch muscles convert more of their pyruvate into lactate via anaerobic fermentation than does slow twitch muscle.

Another possible fate for pyruvate in some types of cells is its conversion to ethanol, via a process called fermentation. In the first step of this reaction, carbon dioxide is removed from pyruvate by the action of the enzyme pyruvate decarboxylase (mechanism from Abeles 186):

Thiamine

Pyruvate decarboxylase contains a tightly bound coenzyme, thymine pyrophosphate. The thymine pyrophosphate helps to stabilize the negative charge on the carbanion.
intermediate resulting from decarboxylation. The product of the reaction is acetaldehyde, which is oxidized by the enzyme **alcohol dehydrogenase** to form ethanol, with corresponding oxidation of NADH to NAD+. So with fermentation, similar to lactate production, the reducing equivalents generated in glycolysis are utilized, creating NAD+ for use by phosphoglycerate dehydrogenase.

Yeasts, which are the major class of organisms which carry out fermentation, produce alcohol anaerobically. However, yeast require oxygen for synthesis of cellular components, and cannot survive for an extended period of time by making alcohol. Yeast seem to produce ethanol to help them compete with other organisms for food. When a fruit ripens, for example, a lot of glucose is produced. Lots of organisms have the ability to generate energy from glucose. However, only a few organisms (which includes the yeast) have the ability to use ethanol as an energy source. Yeast will quickly convert glucose to ethanol as a mechanism to hoard the energy from other organisms.

**REGULATION OF GLYCOLYSIS**

Glycolysis is the first step in the conversion of glucose to energy. Six-carbon sugars are obligated to pass down this pathway to be involved in energy storage. But what happens when our cells have enough energy? We clearly do not want to continue our conversion of glucose to energy; the energy given off by the breakdown of glucose would be wasted as heat. What we would like to do is to **save** some of the energy stored in glucose for when we need it, maybe in the form of glycogen and other storage mechanisms, maybe as glucose-6-phosphate. It's better for the cell to give glucose back to the environment rather than needlessly burn it.

The question then is: how does the cell regulate its glucose consumption?

Much of this regulation occurs in glycolysis. In metabolic pathways, sites of regulation are commonly those enzymes which catalyze essentially irreversible reactions. In glycolysis, the reactions which are most strongly irreversible are those catalyzed by the enzymes **hexokinase**, **phosphofructokinase**, and **pyruvate kinase**. The primary site of regulation in glycolysis is **phosphofructokinase**. Phosphofructokinase as isolated from mammalian liver is a tetrameric enzyme, that is, consisting of four polypeptide chains, with a total molecular weight of around 340,000 daltons. This is an **allosteric** enzyme: ATP acts as an allosteric inhibitor. If you remember allosteric enzymes, they are characterized by this type of kinetic behaviour:
A typical enzyme which obeys Michaelis-Menton kinetics will exhibit a velocity versus substrate curve which is hyperbolic, like this one. In the presence of an allosteric inhibitor, the shape of the curve will change from hyperbolic to sigmoidal. The result of the presence of the allosteric inhibitor is to change the apparent affinity of the enzyme for its substrate. This will require the presence of higher concentration of substrate to achieve a specific reaction velocity; i.e., inhibition.

AMP reverses the inhibitory affect of ATP. Therefore, one measure of the activity of the enzyme phosphofructokinase, and the flux of glucose through glycolysis, is the ATP/AMP ratio. This makes total sense. If most of the adenosine in the cell is in the form of ATP, it doesn't need any more ATP, and therefore doesn't need any more glucose to break down. If the cellular concentration of AMP is relatively high, then this indicates that the cell is in need of energy; the AMP activates phosphofructokinase so that glycolysis can help increase the energy available to the cell.

A second inhibitor of phosphofructokinase is citrate. Citrate is one of the molecules involved in the tricarboxylic acid cycle. In glycolysis, the cellular concentration of citrate provides some measure of the level of precursors available for cellular synthetic reactions. The higher the citrate concentration, the higher the cellular concentration of precursors. Citrate acts as an inhibitor by enhancing the inhibitory affect of ATP.

A final regulator of phosphofructokinase activity is the activator fructose-2,6-bisphosphate. This compound is formed by the action of the enzyme phosphofructokinase 2, and is apparently made when there are high cellular concentrations of fructose-6-phosphate present. Fructose-2,6-bisphosphate activates phosphofructokinase by eliminating the inhibitory effect of ATP on the reaction.

**Fructose-2,6-bisphosphate:**

Last time, I mentioned that the phosphofructokinase was under hormonal control through the molecule fructose-2,6-bisphosphate. Let me elaborate on this a bit before moving on.

**Fructose-2,6-bisphosphate:**

Later in the semester, we will examine the hormonal control of metabolism, but let me begin here by describing the action of the hormone glucagon relative to glycolysis. Glucagon is released in response to low blood glucose, and will bind to receptors on the surface of liver
cells. When glucagon binds to its receptor, it triggers a series of events that lead to the production of cyclic AMP inside of the cell. The cAMP will bind to several protein kinases in the liver cell, activating them. Again, a kinase is an enzyme that transfers a phosphate group either to or from a nucleoside phosphate. One of the kinases triggered by cAMP through glucagon adds a phosphate group to the Phosphofructokinase-2 / Fructose bisphosphatase-2 enzyme. When this enzyme is phosphorylated, the PFK-2 activity is eliminated while the FBPase activity is activated. This will greatly decrease the concentration of F-2,6-BP in the cell, resulting in an inactivation of PFK. This limits the amount of glucose that can be process through glycolysis, allowing more glucose phosphate generated either from glycogen breakdown or from gluconeogenesis (glucose synthesis) to be converted into glucose and released into the bloodstream. Fructose-2,6-bisphosphate is an extremely potent allosteric activator of phosphofructokinase, and an inhibitor of fructose bisphosphatase.

One other side point - don't forget that ATP is also a substrate for phosphofructokinase. It has to be present for the reaction to occur. The point is that in addition to the substrate binding site for ATP on phosphofructokinase there is another ATP binding site not involved in catalysis but in regulation. Think about what the relative affinity of the binding sites for ATP must be in order for this enzyme to work properly.

Phosphofructokinase is the enzyme which directs the flux of glucose through glycolysis. Since the reaction carried out by phosphofructokinase is essentially irreversible, after this reaction occurs the glucose needs to progress through glycolysis to form pyruvate. Before this, glucose-6-phosphate has the option of either being converted to glycogen or being metabolized by a separate pathway called the pentose monophosphate shunt, which we will again speak about in a few weeks. Isomerization by phosphoglucone isomerase has a standard free energy change of near zero and is completely reversible. Since phosphofructokinase carries out the committed step in the reaction, it then makes sense that it is the primary point of regulation.

Two other enzymes in glycolysis which are important in pathway regulation are hexokinase and pyruvate kinase. Hexokinase is inhibited by glucose-6-phosphate - product inhibition. Product builds up when phosphofructokinase is inhibited. In most cells, excess glucose is not phosphorylated but instead is allowed to diffuse back into the cellular
environment. However, liver has another enzyme, glucokinase, which catalyzes the conversion of glucose to glucose-6-phosphate. Glucokinase has a much lower affinity for glucose ($K_M$) than hexokinase, so this enzyme is only used in times of high energy supply. The liver can use excess glucose-6-phosphate to form glycogen, which we'll talk about in a few weeks.

Pyruvate kinase is the third enzyme important in regulation of flux through the glycolytic pathway. Pyruvate kinase is a tetrameric enzyme, and is allosterically inhibited by ATP and alanine. Alanine can be synthesized from pyruvate in one step, and therefore provides a measure of how available cellular synthetic precursors are at the time. The enzyme is activated by fructose-1,6-bisphosphate, enabling the pathway to process intermediates if their concentrations are high enough.

Finally, the activity of pyruvate kinase can be modified by glucagon action. Glucagon can react with cells and cause the reversible phosphorylation of pyruvate kinase in the liver. Hormonally induced phosphorylation decreases the activity of the liver enzyme relative to brain and muscle pyruvate kinase. The brain uses glucose almost exclusively as an energy source; glucagon is released when the blood glucose is low. The function of glucagon is to slow the liver's consumption of glucose so that more will be in the bloodstream for consumption by the brain.

2,3-bis-phosphoglycerate

Going back to glyceraldehyde-1,3-bisphosphate, there is a second reaction which can occur. Glyceraldehyde-1,3-bisphosphate can be converted into glyceraldehyde-2,3-bisphosphate by the action of the enzyme Bisphosphoglycerate mutase:

The reaction requires the participation of one 3-phosphoglycerate. 2,3-Bisphosphoglycerate has an important cellular function in red blood cells, where it acts as an allosteric inhibitor of oxygen binding. Its concentration can reach to about 4 mM in red blood cells. 2,3-Bisphosphoglycerate is also an important intermediate in the reaction catalyzed by phosphoglyceromutase. This enzyme has a phosphorylated histidine residue in its active site. 3-Phosphoglycerate is phosphorylated to form 2,3-bisphosphoglycerate; the phosphate group from the 3-position is then transferred back to the active site histidine.

**ENTRANCE OF ALTERNATIVE SUGARS INTO THE GLYCOLYTIC PATHWAY**
We now have some idea on how glucose begins to be metabolized in cells. How about other commonly occurring sugars? Let's look at two of these, fructose and galactose.

Fructose may enter metabolism by phosphorylation of fructose at the 1 position by the enzyme fructokinase, via the reaction:

Fructose-1-phosphate can be broken into two three-carbon compounds by the action of the enzyme fructose-1-phosphate aldolase, where the reaction is:

The final products of the fructose-1-phosphate aldolase are glyceraldehydes and dihydroxyacetone phosphate, which is a glycolytic intermediate. It can enter the glycolytic pathway after conversion to glyceraldehyde-3-phosphate via triose phosphate isomerase. The glyceraldehyde needs to be phosphorylated before entering; this is carried out by the enzyme triose kinase, in the reaction:

How does the net energy production with fructose compare with the net energy production from glucose? Irregardless of whether the first step is phosphorylation in the 6-position by hexokinase or in the 1 position by fructokinase, the net energy production is the same - 2 ATP need to be expended per fructose molecule to result in 2 phosphorylated 3-carbon units.

Getting galactose into the glycolytic pathway is a little more difficult. Galactose has to be converted into glucose-6-phosphate; this occurs in four steps. The first step is phosphorylation of galactose at the 1-position by the action of the enzyme galactokinase:

The galactose-1-phosphate then acquires a uridyl group from UDP-glucose. Let me draw out the structure of this group for you on the blackboard:

The UDP-sugar molecule turns out to be important in several other series of reactions which will be described later in the course. The above reaction is catalyzed by the enzyme galactose-1-phosphate uridyl transferase.

After the UDP group is attached to the galactose, the galactose sugar can be epimerized to glucose by action of the enzyme UDP-galactose-4-epimerase. The enzyme has this name because glucose and galactose are epimers; they differ in configuration at a single position, that is, the C-4 position on the sugar. The reaction is:

So UDP-galactose is converted to UDP-glucose, which was used up in the formation of UDP-galactose. What is left over? The glucose-1-phosphate. Glucose-1-phosphate is converted
Disaccharide metabolism

Since we consume a great deal of these three sugars, it is important that we know how we metabolize three different disaccharides: maltose, lactose, and sucrose. Maltose is a disaccharide consisting of two glucose residues linked by an \( \alpha-1,4 \)-glycosidic bond. Lactose is galactose-\( \beta-1,4 \)-glucose, and sucrose is glucose-\( \alpha 1,\beta-2 \)-fructose. These are broken down into their individual monosaccharides by intestinal enzymes: mannase breaks apart mannose; lactase breaks lactose, and sucrase breaks sucrose.

Lactose intolerant individuals lack intestinal lactase. As a result, intestinal lactose builds up and is consumed by intestinal bacteria, who proliferate and make a huge mess. Lactose deficiency can be overcome by eating a pill containing the digestive enzymes, or simply by avoiding lactose, which is commonly found in milk products.

Glycerol is a component of fat that we eat; it is a three carbon alcohol. It is brought into glycolysis first by phosphorylation by glycerol kinase, followed by the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase.

Polysaccharide metabolism

Starch metabolism

Your textbook describes polysaccharide metabolism in two stages: starch metabolism and glycogen metabolism. Starch, or amylopectin, is a branched polymer of glucose monomers that are connected by \( \alpha-1,4 \) and \( \alpha-1,6 \) glycosidic bonds. Starch is digested in the mouth, stomach, and intestinal track by the enzyme amylase into glucose and maltose. Amylase does not break \( \alpha-1,6 \) glycosidic bonds; these are broken by the enzyme \( \alpha-1,6 \) glucosidase. Maltose is broken down into glucose by maltase.

Glycogen metabolism

How is glycogen metabolized to release free glucose residues? As with other pathways, the metabolism of glycogen is not the reverse of the synthetic process. One glucose residue is removed from glycogen by breaking an \( \alpha-1,4 \) linkage and adding inorganic phosphate back to the 1-position on the glucose. This reaction is carried out by the enzyme phosphorylase:

Phosphorylase catalyzes the addition of a phosphate group to the glucose residue
hydrolyzed from glycogen. This is energetically advantageous over first hydrolyzing to glucose and then phosphorylating; this would cost the cell an ATP.

Phosphorylase likely works using a general acid/base mechanism. Phosphorylase has an active site pyridoxyl phosphate residue; this probable serves as a proton donor and acceptor. The mechanism of phosphorylase likely involves the formation of a carbanion intermediate at carbon 1 of glucose.

Phosphorylase is used to break α-1,4 glycosidic bonds in glycogen. To break α-1,6 bonds, a different enzyme system is used. The required substrate for breaking the α-1,6 bond is a glycogen chain with a branch point, with four glucose residues remaining on each side of the branch point. Therefore, the substrate representationally looks like this:

The debranching process begins by the action of an enzyme called a $\alpha$-1,4Æ$\alpha$-1,4 glucantransferase. The transferase removes the 3 glucose residues from the end of the branch point held by the $\alpha$-1,6 linkage and transfers them to the end of the branch held by the $\alpha$-1,4 or $\alpha$-1,6 linkage. The result of this transfer is a long amylose chain with one glucose connected by an $\alpha$-1,6 linkage. Once this condition is set, the enzyme $\alpha$-1,6-glucosidase (which is the same enzyme, just a different reaction!) hydrolyzes the glycosidic bond, removing glucose and leaving the long chain behind.

The transferase function and the glucosidase function are all part of a single polypeptide chain. The total enzyme is called debranching enzyme.

How are glycogen synthesis and glycogen metabolism regulated? Let's look first at glycogen metabolism. Phosphorylase is primarily regulated by specific phosphorylation and dephosphorylation events at regulatory sites on phosphorylase. These actions are primarily regulated by the actions of hormones on the cell. I want to delay talking about hormones in any detail until the end of the semester, because I want to talk about all of the actions of a specific hormone and how these work together to achieve a metabolic goal. However, I do want to talk just a bit on phosphorylase regulation.

Let's work with one fairly well understood hormone, which is glucagon. Glucagon is secreted when blood glucose is low. The goal of glycogen stimulation muscle is to promote gluconeogenesis and glycogen metabolism, which makes the liver cell less dependent upon foreign glucose, allowing it to go to the brain or other sites of need.

How does glucagon action result in glycogen metabolism? Let's first go back to talk
about phosphorylase. In the muscle, phosphorylase exists in two forms, phosphorylase b and phosphorylase a. Phosphorylase b is a dimeric protein, with each subunit having a molecular weight of about 97 kd. Phosphorylase b is allosterically regulated by ATP, AMP, and glucose 6-phosphate. It is only active when the AMP/ATP ratio is high, or glucose 6-phosphate is low; that is, the enzyme is sensitive to the energy needs of the cell.

Phosphorylase a is the same dimeric protein as b, except that each of the polypeptide chains has a covalently bound phosphate group at serine-14. This phosphorylated phosphorylase is no longer regulated by the energy supply in the cell. It is active only unless the concentration of free glucose in the cell is high. OVERHEAD

The phosphorylation of phosphorylase is catalyzed by a specific protein called phosphorylase kinase. Phosphorylase kinase is activated by phosphorylation and also by high concentrations of calcium ions. Phosphorylase kinase is in turn phosphorylated by protein kinase A. Protein kinase A is activated by a small molecule called cyclic-AMP. Cyclic AMP is made by the interaction of a hormone with the liver cell; in this case, glucagon. The hormone causes formation of cyclic AMP, which activates a specialized protein kinase A.

The same process occurs in muscle, except that the cascade is triggered by the hormone epinephrine. Muscle does not contain the enzyme glucose-6-phosphatase, and therefore muscle glycogen cannot be used to provide glucose when blood glucose is low. However, glycogen stores in muscle can be mobilized when the muscle needs more glucose energy. Epinephrine is released in response to some stress; the glucose generated allows the muscles to better respond to the stressful situation.

We'll discuss later in the semester all about cyclic AMP formation and removal, hormone receptors, all that good stuff.

**Glycogen storage diseases**

I've taken the time to photocopy the pages in Voet which describe the different types of glycogen storage diseases which are found in the population. The most common type of glycogen storage disease, observed with a frequency of about 1 in 200,000, is **Type I glycogen storage disease or von Gierke’s disease**. This disease is caused by a deficiency in liver, intestinal mucosa, and kidney glucose-6-phosphatase. Suppose that we are hypoglycemic - that our blood sugar is too low. A message is sent to the liver to release glucose. The fastest way for the liver to release glucose is to break glycogen into glucose-1-phosphate through
phosphorylase, which is isomerized to glucose-6-phosphate. The phosphate group on glucose-6-phosphate is removed by the action of the enzyme glucose-6-phosphatase:

If this enzyme is deficient or missing, the liver cannot break down glycogen to make glucose. Also, without glucose-6-phosphatase, there can be a buildup of glucose-6-phosphate, which inhibits phosphorylase and results in a buildup of glycogen in the liver. The patient with von Gierke’s disease is characterized by an enlarged liver due to overproduction of glycogen, hypoglycemia, and “a general inability to thrive”. Treatment, in order of severity, are:
1. Provide carbohydrate throughout the day to keep blood glucose levels at a constant level and avoid the formation of glucagon, insulin, and epinephrin, which would affect glycogen metabolism in the liver.
2. Surgical transposition of the portal vein, which allows glucose-rich blood from the intestine to bypass the liver on the first pass and be distributed to the tissues. This gives an increased role in blood glucose maintenance and carbohydrate metabolism to tissues other than the liver.
3. Liver transplant.

The next most prevalent glycogen storage disease is **Type II, or Pompe’s disease**. In this disease, the enzyme α-1,4-glucosidase (or maltase) is missing from lysozomes. Cells normally take up glycogen granules and metabolize them in lysozomes. However, lacking the maltase, the glycogen is not metabolized, making the lysozomes inactive and creating general havoc in the cells. The result is glycogen buildup in every cell; the patients develop cardiomegaly and dye usually before the age of one year old.

**Type III glycogen storage disease, or Cori’s disease**, is caused by a deficiency of glycogen debranching enzyme. The clinical manifestations of the disease are similar to that seen for Type I deficiency, but much less severe.

**Type V glycogen storage disease, or McArdle’s disease**, is caused by an absence of muscle phosphorylase. Glycogen cannot be broken down in these cells, although it can be synthesized. The disease is characterized by muscle cramps and an inability to perform exercise, presumably because muscle glycogen stores are not available to the exercising muscle. There is no treatment for McArdle’s disease save for rest and avoiding exercise.