Enzymes and Metabolic Pathways

“Un-lecture!”

Numbers correspond to the slides, which are in your lecture notes and also posted on-line on the announcements page.

1. Characteristics of enzymes….we went over these in class!

2. Catalysts: Notice that catalysts speed up reactions. They can be organic (like our enzymes) or inorganic (like “rust” (Fe₂O₃). In both cases, the catalyst increases the reaction by providing a surface on which the reactants can meet.

3. Enzymes are organic catalysts: Because hydrogen peroxide (H₂O₂) is toxic to cells, cells must have a way to neutralize or isolate the hydrogen peroxide to prevent cellular damage. One enzyme that can “neutralize” the hydrogen peroxide is catalase. The liver, as the detox center of the body, has an abundance of catalase in its cells. This enzyme speeds up the break down of H₂O₂ into H₂O and O gas.

4. Graphs comparing catalyzed and noncatalyzed reactions. Look at the two graphs at the bottom of the slide. They show that catalyzed reactions require less activation energy.

5. Mechanism of enzyme action: The shape of the enzyme and its active site are responsible for the decrease in activation energy. They orient molecules in such a way that reactions occur more quickly with less energy expended.

6. Lock and Key Model: This models says that the active site of the enzyme is the perfect fit for the substrates it interacts with. (Substrate is just a name for any molecule on which an enzyme acts).

7. Induced fit model: This model says that one substrate fits into the active site, and causes a shape change that makes it possible for another substrate to also enter. Therefore, the first substrate “induces” the new shape to accommodate the second substrate. Both of these can occur in enzyme substrate interactions.

8. Enzymes accelerate….: visually compares the two models.

9. Cofactors and coenzymes: Cofactors are trace elements and coenzymes are a type of cofactor that is derived from vitamins. Some enzymes require a coenzyme or cofactor to create the right shape in the active site.
so that the enzymes are more effective in catalyzing the reactions. Did you notice that all of these are charged ions or molecules? Adding those charged ions or molecules affects the shape of the enzyme. This is one of the reasons why you need vitamins and minerals in your diet!

10. Reaction rate: What effects how fast an enzyme mediated reaction can go? **Temperature** (because higher temperature increases the rate of movement of molecules. Initially you get an increase in rate...but what happens to the enzymes if the temperature gets too high? The enzyme will denature and the reaction slows down!) **pH** (because pH effects the shape of the enzyme...there is an optimal pH, which is the pH at which an enzyme works best. In the stomach, the pH is about 1.5-2. This is the “best” or optimal pH for enzymes in the stomach. Other enzymes work best at a pH of 7.35-7.45, and can be denatured if the pH changes dramatically.) **cofactors and coenzymes** (the concentration of these can effect an enzyme mediated reaction if the enzyme requires cofactors and coenzymes. You then need enough of these to enhance the function of those enzymes, which allows the reaction to go more rapidly). **Enzyme an substrate**: (the concentration of enzyme and substrate needs to match..ie, if you don’t have enough enzymes, then the substrates have to “wait” for a chance to occupy the active site. This slows things down. If you don’t have enough substrate, then you can’t make more product.) **Stimulatory and inhibitory effects**...(Some products made in enzyme mediated reactions can go back and act on enzymes to either slow them down or speed them up).

11. **Effect of temperature**: slide is self-explanatory.

12. **Effect of pH**: slide is self-explanatory.

13. **Effect of substrate**: slide is self-explanatory.

14. **Controlling enzyme activity**: Enzymes work on the basis of their 3-D shape. Therefore, if you change the shape, you alter the function of the enzyme. You already know that when the shape of an enzyme is permanently altered, the enzyme is denatured and no longer functions. However, there are many enzymes that your body makes that need to be around so that they are available when they are needed, but aren’t needed all the time. So, we have to have a way to turn these enzymes on when we need to use them, and off when we don’t. We do this by the first three methods: allosteric modulation, covalent modulation, and end-product inhibition. You already know that denaturation is a “one-way trip”. Once an enzyme is denatured, it can no longer be used.

15. **Allosteric modulation**: Enzymes that are regulated by allosteric modulation have two sites: the active site, which interacts with the
substrate, and a regulatory site, where an ion is added that regulates the shape of the active site. In allosteric modulation, an ion or charged molecule is added to the regulatory site by forces other than covalent bonds, causing a shape change in the active site that either turns on or turns off the enzyme temporarily. In the image, the enzyme is the wrong shape for the substrate until you add the Na+. With Na+, the shape of the active site is changes so that the substrate fits.

16. Covalent modulation: In this type of enzyme regulation, the enzyme has an active site where the substrate interacts, but no special regulatory site. In covalent modulation, a charged molecule is added to an enzyme by covalent bonds, causing a change in the shape of the active site that either turns on or turns off the enzyme. The charged molecule that is most common in this type of an event is phosphate (PO$_4^-$). Adding a phosphate to a molecule is called phosphorylation. You can see that this molecule has a strong negative charge and it has a dramatic effect on the shape of the enzyme. This type of modulation is much more complex than allosteric modulation. In fact, to add the phosphate to the enzyme requires another enzyme called a protein kinase. To remove the phosphate from the enzyme requires a different enzyme called a phosphoprotein phosphatase. Both of these enzymes are allosterically modulated!

17. Metabolic pathways: Many substances in the body are produced as a results of a long series of chemical reactions called metabolic pathways. These reactions utilize several different enzymes at each step, producing intermediate products, on their way to the final product.

18. End product inhibition: As final products are produced in metabolic pathways, they are used. However, as more and more are made, they can also begin to accumulate, if there are more being made than are needed. Some of these can go back and bind to one of the enzymes in the metabolic pathway, altering the shape of that enzyme and slowing the pathway down. This enzyme is at the "rate limiting step", because when the endproduct binds to it, it slows the reaction down and decreases the amount of the final product that is made. As all of the available product is used, the endproduct comes off of the enzyme, and the reaction speeds up again. This is a way of "self regulating" the amount of product that is produced so that the body does not waste materials or energy making something that isn’t needed at the time.

19. End product inhibition continued: Read this and pay attention to the term “feedback inhibition”.

20. Inborn errors of metabolism: Slide is self explanatory. This shows what can happen when a defective gene produces an enzyme that does not work.
21. **Denaturation**: Slide is self explanatory. Think of the lab demonstration with the liver and egg white.

22. **Bioenergetics**: This slide describes how energy moves through living systems and describes the first and second laws of thermodynamics. Read slide, self explanatory.

23. **Endergonic and exergonic reactions**: If you recall in our discussions, when bonds are produced, energy is captured and the reaction is said to be endergonic. Because the energy goes into the reaction, the products will have more energy than the reactants that made them. In Exergonic reactions, bonds are broken and energy is released. Therefore, the products have less energy than the reactants.

24. **Coupled reactions**: We mentioned that reactions in living systems are coupled to prevent the waste of energy. When bonds are broken and energy is released (exergonic reactions), that energy is captured in an energy transfer molecule (ATP) and taken to another reaction (endergonic) in which it is used to make products. So ATP is the link between coupled reactions.

25. **Formation of ATP**: This slide describes how ATP is formed and shows you again the chemical structure of ATP. It is self explanatory. Note: ATP = **adenosine triphosphate** = adenosine + 3 phosphates, ADP = **adenosine diphosphate** = adenosine to 2 phosphates, AMP = **adenosine monophosphate** = adenosine to 1 phosphate. The bonds between phosphates are called high energy bonds because they are able to hold more than the amount of energy in an ordinary bond.

26. **Oxidation-reduction reactions**: These reactions are also coupled, but in this case one molecule is oxidized (looses electrons) and another molecule is reduced (gains electrons). Sometimes, H⁺ is also transferred. Coenzymes like NAD⁺ and FAD⁺ carry H⁺.

27. **NAD⁺ and FAD⁺**: This image shows what happens structurally when NAD⁺ (oxidized) becomes NADH (reduced), and FAD⁺ (oxidized) becomes FADH₂ (reduced).

28. **Aerobic cellular respiration**: In this section, we investigate how energy is made inside the cell. When you eat, the food that you eat is broken down, digested, and absorbed into your body in the form of simple sugars, amino acids, etc. If you recall in our discussion of what these things do in the body, simple sugars are used for energy. But what happens to those sugars once they get across the cells of the small intestines and into your blood? How do they provide energy for the cells? This section describes
what happens. The sugar (glucose) is absorbed into each cell in your body with the help of insulin, which is secreted by the pancreas. Once the sugar gets into your cells, it is broken down to release energy which is used to drive the metabolic reactions in the cell. This process takes place in 3 major steps, although I divide it up into 4. The first step is **glycolysis**, which takes place in the cytosol of the cell after the glucose has come in. The next step is the **intermediate step**, which occurs as the products of glycolysis move into the mitochondria. The next step is called the **Krebs cycle**, which occurs inside the space within the folds of the inner mitochondrial membrane. That center space is called the matrix. And finally, ATP is produced from other energy transfer molecules in the electron transport system, or **ETS**, on the cristae of the mitochondria. This is called aerobic cellular respiration because it requires oxygen to complete the process. We will now look at each of these steps individually. To keep track of the events, it helps to produce a table like the one in this presentation called **Summary of aerobic cellular respiration**. As you go through the steps, look at this table and used it as a guide to help you understand what is happening. Remember: the goal is to take glucose and break the bonds so that the energy trapped in those bonds is released, transferred to ATP, and can be used by the cell to make other things that it needs.

**29. Glycolysis:** The first sentence is self-explanatory. Note that oxygen is not required for this first step in cellular respiration. Glycolysis is anaerobic, which means it does not require oxygen. Glycolysis begins when a molecule of glucose enters the cell. Glucose, a six carbon sugar, is broken down through a series of reactions resulting in the production of two three carbon molecules called pyruvic acid, or pyruvate. As bonds are broken in this series of reactions, two types of energy transfer molecules are made: ATP and NADH+H⁺ (which I sometimes refer to in this section as NADH or NADH₂).

**30. Overview of glycolysis:** This slide provides a visual summary of what happens in glycolysis. Pay attention to the number of carbons in each molecule, and the name and number of all of the energy transfer molecules produced. Notice also that there is some “activation energy” needed to get this reaction started. Before anything can happen, two ATP are used (these are shown in purple with arrows pointed in, rather than out). So it “costs” energy to make the energy the cell needs. When glycolysis is completed, in addition to the 2 pyruvic acid, you will also have produced 4 ATP (2ATP net, because you had to “spend” two to get the reaction started) and 2 NADH.

**31. Glycolysis in detail, part I:** Notice that there are green numbers next to each step so that you can keep track of what happens. You do not need to know the names of all of these intermediary molecules, but I think it is
important for you to see them so that you understand what is happening.
You will notice first of all that as glucose comes into the cell, one ATP is
converted to ADP, releasing energy, and the phosphate that is removed
from the ATP is added to the glucose at carbon number 6. Now the
molecule is called glucose -6-phosphate. The significance of this is that
the glucose molecule is no longer considered to be glucose when you are
looking at the diffusion gradient. So, glucose is always higher outside the
cell than inside, and this drives the movement of glucose into the cell.
Secondly, once you add the phosphate to the glucose, it is now charged
and it can’t get back out through the plasma membrane. In step 2, the
glucose-6 –phosphate is converted to fructose-6-phosphate. Remember
that glucose and fructose are isomers (they are made up of the same
molecules, but organized a little differently). Another phosphate is then
added when the second ATP is converted to ADP, producing fructose-1,6
biphosphate (this means one phosphate is attached to carbon #1 and the
second phosphate is attached to carbon #2).

32. Glycolysis in detail, part II: Now the fructose 1,6 biphosphate splits into 2
molecules of PGAL (phosphogyceraldehyde). The PGAL is converted to 2
molecules of 1,3 biphosphoglyceraldehyde. When this occurs, energy is
released and captured by a different type of energy transfer molecule
called NAD+. When the energy is added, this molecule is reduced and
becomes NADH. It helps to think of NADH as “foreign currency” and ATP
as “US dollars”. Let’s pretend that NADH is Euro’s. Euro’s are money,
but they can’t be used to buy things in the US. You have to take the
Euros to the bank and exchange them for US dollars. So, before the cell
can use the energy in the NADH, it has to be converted to ATP. This will
happen later in the electron transport system (ETS). For now, let’s just
keep track of how many of these are made. The next step converts 1,3
biphosphoglyceraldehyde to 2 molecules of PGA (phosphoglyceric acid),
producing another 2 ATP (notice that this time ADP is converted to
ATP...bonds are being made and energy is captured). The PGA is then
converted to 2 molecules of PEP (phosphoenolpyruvate) and finally, the
PEP is converted to the endproduct of glycolysis, 2 molecules of pyruvic
acid producing another 2 molecules of ATP.

33. With Oxygen: If oxygen is present, then the process can continue and the
pyruvic acid will go to the intermediate step. This is necessary because
the next major event, the Krebs cycle, can only receive two-carbon
molecules. We just ended glycolysis with 2 molecules of pyruvic acid,
which each have 3 carbons. So, we need to remove a carbon from each
of these to be able to go into the Krebs cycle. The process of removing
carbons is called decarboxylation. When we remove the carbon from the
pyruvic acid, it becomes acetyl, a 2 carbon molecule. When that bond
breaks, it releases energy which is captured by NADH. The carbon that is
removed leaves our bodies as CO₂ (that’s where some of the CO₂ we
exhale comes from!) The two acetyl molecules that are made when the carbon is removed from pyruvic acid will bind to a special carrier called CoA and are now called acetyl CoA. The CoA will take the acetyl to the Krebs cycle and drop it off, then go back to pick up more acetyl. If there is no oxygen present, then we cannot make any more energy at this point and we complete the process with fermentation. Therefore, our only task now is to remove the electrons from the NADH that we made in glycolysis. This is necessary because NADH is made from a type of vitamin B. Vitamin B is water soluble, and can’t be stored in the body. We have to remove the electrons from NADH, converting it back to NAD+ so that we can continue to go through glycolysis. The electrons from the NADH have to go somewhere, and in this case, pyruvic acid becomes the final electron acceptor. Two possible products can be made, either ethanol (alcohol) and CO₂, or lactic acid.

34. Intermediate step: visual representation of what happens as described in previous slide.

35. Fermentation: shows the lactic acid pathway

36. Lactic Acid Pathway in humans: When we do not have sufficient oxygen present, or when cells don’t contain mitochondria (remember that mature red blood cells get rid of their nucleus and organelles as they develop), they can use the lactic acid pathway to make energy. They do not make as much energy as you would if you complete the process, but some is better than none. When your ability to bring in oxygen falls behind the amount you need for normal skeletal muscle activity, your muscle will start to make and accumulate lactic acid. We call this oxygen debt. The lactic acid can be converted back to pyruvic acid when enough oxygen is present and can complete cellular respiration. Your cardiac muscle, however, needs oxygen and if it is functioning anaerobically, the result is myocardial ischemia and angina pectoris (pain). It can ultimately lead to a myocardial infarction (MI = heart attack) if the heart muscle is deprived of oxygen too long and starts to die.

37. Fermentation summary: self explanatory. Notice that you only produce 2 ATP when you have to use this pathway! Compare this (later) to how much you would make if you go all the way through the ETS and complete cellular respiration.

38. Krebs Cycle: This gives the main points. Because the Krebs cycle can only receive one 2-carbon acetyl at a time, you have to go through it twice for every glucose you start with (you make 2 acetyl, remember?) By the time you finish, all of the carbon bonds have been broken and all you have is CO₂, water, and energy transfer molecules. There are three energy
transfer molecules involved: GTP (which is the same as ATP, but uses guanine instead of adenine), NADH, and a new one called FADH$_2$.

39. Krebs Cycle: Overview: A “cycle” always begins and ends with the same molecule. The Krebs cycle begins and ends with Oxaloacetic acid (OAC), a 4 carbon molecule. When the acetyl comes in, it binds with the OAC to form citric acid, a 6 carbon molecule. In fact, sometimes the Krebs cycle is called the citric acid cycle because of this. Now we need to break the bonds and restore the OAC so that the cycle can continue. You can see that in one turn of the Krebs cycle, we produce 1 GTP, 3 NADH, and 1 FADH$_2$. We also produce 2 CO$_2$. So, for two acetyl, you produce 2 GTP, 6 NADH, and 2 FADH$_2$. Now all of your glucose has been broken down.

40. picture. This is just a slide showing the molecules involved in Krebs cycle.

We will end here for now and I will post a second lecture for you on Wednesday or Thursday of next week covering the rest of this presentation. Good luck and see you on Wednesday or Thursday at bootcamp!