Objectives for Test Three: Chapter 6 and 33 (33.2 - 33.5):

Metabolism, ATP, Enzymes and Digestion

You should be able to:

1. Distinguish between anabolic and catabolic metabolic pathways.
2. Explain the relationship between kinetic energy, potential energy & thermodynamics.
3. Explain the relationships among enthalpy (energy), entropy (disorder), changes in free energy, spontaneous and non-spontaneous reactions, endergonic and exergonic reactions, downhill and uphill, negative and positive changes in free energy (ΔG), respiration and photosynthesis.
4. Discuss the meaning of the equation: ΔG = ΔH - TAS.
5. List the three main ways cells do work.
6. Describe the structure of ATP. Explain why “high energy phosphate bonds” is misleading.
7. Explain the ATP cycle.
8. Explain how ATP supplies energy to an endergonic reaction (e.g. discuss phosphorylation).
9. Describe the effect enzymes have on chemical reactions.
10. Describe the relationship between enzyme, substrate and active site.
11. Sketch and label a graph illustrating the relationship between the free energy of reactants and products, activation energy with and without enzymes, and the ΔG of a reaction.
12. Describe four different mechanisms by which enzymes can lower activation energy.
13. Distinguish between “lock and key” and “induced fit” models of enzyme action.
14. Provide examples and describe the role of cofactors and coenzymes in enzymatic activity.
15. Describe the effect of enzyme inhibitors (competitive and non-competitive) on the rate of reactions catalyzed by an enzyme. Give examples of real inhibitors.
16. Relate the terms from the previous objective to hydrogen peroxide and liver.
17. Identify the characteristics of enzymes.
18. Given data, construct graphs and then calculate the rate of a reaction catalyzed by an enzyme.
19. Sketch a graph to illustrate the effect that changing temperature, enzyme concentration, substrate concentration, pH, salinity, inhibitor concentration will have on the rate of a reaction catalyzed by an enzyme. Explain why each graph takes the shape it does.
20. Explain how feedback inhibition can regulate an enzyme and how it’s location in the cell or membrane and increase it’s efficiency. (We did not talk about his in class – pg. 159)
21. Distinguish between filter feeders, fluid feeders, and bulk feeders. Explain the problem a bulk feeder has that a fluid feeder does not.
22. Identify the organs of the alimentary canal and the accessory glands of the human digestive system. Label a diagram of the human digestive system, such as that in figure 41.10.
23. Describe the mechanical and chemical digestion in each of the major organs of the alimentary canal.
24. Describe adaptations, both physical and chemical, of the human digestive system that enhances the functioning of the digestive enzymes.
25. Discuss the relationships among pepsin, pepsinogen, HCl, mucus, acid-tolerant bacteria, and gastrin.
26. Describe adaptations that prevent inappropriate digestion from happening.
27. Identify the substrate, original production location, and the end product of the following human digestive enzymes:
   a. amylase
   b. pepsin
   c. lipase
   d. Nucleases
28. Describe how the structure of the small intestine enhances absorption of the products of digestion.
29. Describe how the large intestine functions to retain the body’s liquids.
30. Compare the size of a cecum in a carnivore with that of an herbivore.
31. Describe the digestive system of a ruminant such as a cow. How can a cow eat grass when it can’t digest cellulose?
32. Describe how a hydra ingests its food.
33. Explain how termites can eat wood.
34. Regarding nutrition, explain:
   a. essential amino acids / fatty acids
   b. complete vs. incomplete proteins
   c. undernourishment / overnourishment / malnourishment
35. Explain the relationship between enzymes and vitamins and minerals. Give such examples of vitamins and minerals, their primary function and problems if levels are too low:
   a. Vitamin B6
   b. Vitamin A
c. Vitamin D  
d. Calcium  
e. Iron  
f. Iodine

36. Regarding our LABS on enzymes:
   a. Given a graph of a reaction catalyzed by an enzyme calculate the rate (initial velocity) of the 
      reaction.  
   b. Design a controlled experiment to test the effect of an abiotic or biotic variable (pH, temperature,  
      salinity, enzyme concentration, substrate concentration, inhibitor concentration, enzyme source)  
      on the rate of a reaction catalyzed by an enzyme.  
   c. Explain why pressure could be measured to get at the rate of hydrogen peroxide breakdown by 
      catalase. (Be familiar with this procedure)  
   d. Explain why a spectrophotometer could be used to get at the rate of hydrogen peroxide 
      breakdown by peroxidase. (Be familiar with this procedure)

37. Each chapter has some multiple choice questions and a few other additional questions at its end. Give 
these a try. You might see them again!

Some Examples of Short Free Response Questions: (2-3 sentences): These might be the actual questions.

1. Relate ΔG to activation energy (E-A) to exergonic (exothermic) and endergonic (endothermic) reactions.  
2. Relate the tertiary and/ or quaternary structure of a protein enzyme to the specificity of an active site and  
   the corresponding substrate.  
3. How do competitive inhibitors work in relationship to the active site? What do non-competitive  
   inhibitors, co-factors, and co-enzymes share in common?  
4. Scientists try to design enzymes to work better in commercial applications (Tide detergent, biofuels).  
   What do scientists need to consider in designing these enzymes?  
5. Identify TWO environmental factors that can change the rate of an enzyme-mediated reaction. Discuss 
   how each of those two factors would affect the reaction rate of an enzyme.  
6. Given a graph of a trial for alpha-amylase, draw and label another line on the graph to predict the 
   results if the concentration of alpha-amylase was doubled. Explain your predicted results.  
7. Given some data, graph the data on the axes provided and calculate the rate of the reaction over a given 
   time period.  
8. Describe the structure of the ATP  
9. Discuss how the structure of a protein affects the regulation of enzyme activity.  
10. The relationship of structure to function is one of the major themes in biology. Describe the structure of 
    an enzyme and explain how this structure is related to the function of catalysis.  
11. Using pepsin as an example, explain several ways the digestive system protects itself of self-digestion.  
12. Explain two ways the activity of enzymes are regulated in cells.  
13. Describe the processes of fat digestion and product absorption in the small intestine. Include a 
    discussion of the enzymatic reactions involved.  
14. Describe the processes of protein digestion as it occurs in the human stomach. Include a discussion of 
    the enzymatic reactions involved.  
15. Describe two adaptations of two different organisms that enhance their ability to digest and absorb 
    nutrients.  

The Essay to write outside of class is #145: (This will be the last one you write outside of class).

The effects of pH and temperature were studied for an enzyme-catalyzed reaction. The following results were obtained.

![Graphs showing enzyme activity vs. temperature and pH](image)

a) How do (1) temperature and (2) pH affect the activity of this enzyme? In your answer, include a discussion of the 
   relationship between the structure and the function of this enzyme, as well as a discussion of how structure and 
   function of enzymes are affected by temperature and pH.

(b) Describe a controlled experiment that could have produced data shown for either temperature or pH. Be sure to 
   state the hypothesis that was tested here. (You may identify a specific enzyme enzyme and use it in your 
   experiment.)
AP Biology: Metabolism, ATP And Enzymes

Energy released Energy supplied
Product
Reactant
Energy must be supplied.

Reactant

Energy is released.
Product

(a) Gravitational motion
(b) Diffusion
(c) Chemical reaction

Copyright © 2009 Pearson Educación, Inc. y Her Majesty the Crown in Right of Canada.
AP Biology: Metabolism, ATP, and Enzymes

Adenosine triphosphate (ATP)

(a) Endergonic reaction

\[ \text{Glu} + \text{NH}_3 \rightarrow \text{Glu} + \text{NH}_2 + \text{Energy}, \quad \Delta G = +3.4 \text{ kcal/mol} \]

(b) Coupled with ATP hydrolysis, an exergonic reaction

\[ \text{Glu} + \text{NH}_3 \rightarrow \text{Glu} + \text{NH}_2, \quad \Delta G = +3.4 \text{ kcal/mol} \]

\[ \text{ATP} \rightarrow \text{ADP} + \text{P}_i, \quad \Delta G = -7.3 \text{ kcal/mol} \]

Net \( \Delta G = -3.9 \text{ kcal/mol} \)

(c) Overall free-energy change

Adenosine diphosphate (ADP)
AP Biology: Enzyme Diagrams
AP Biology: Enzyme Diagrams

(a) Normal binding

(b) Competitive inhibition

Allosteric inhibitor changes shape of enzyme so it cannot bind to substrate

(b) Noncompetitive inhibition
Chemical Reactions and Energy

The following is a tutorial on the relationship between chemical reactions and energy. This is really important stuff! You must do each question for the next one to make sense. The questions build on each other and require you to apply each one to the next. You may use resources (textbook and internet) for definitions, but the majority of the questions require to THINK and apply the information given and what you already know in order to answer them.

1. State the first law of thermodynamics.

2. What is chemical energy? Explain where the energy is in a molecule. How could energy be added to a molecule? How could energy be released from a molecule?

3. The first law of thermodynamics applies to all energy transformations including chemical reactions. This means that a chemical reaction can release or absorb energy, but it can never create or destroy energy. What would happen if a chemical reaction released energy? How might you be able to tell that a chemical reaction released energy?


5. You can think of a chemical bond sort of like two magnets sticking together. The positive pole of one magnet is attracted to the negative pole of the other. If you move them next to each other they will naturally stick together (it won’t require any energy to get them to do so). Once the magnets are joined it would require energy (the ability to do work = a force applied over a distance) to separate them.

Using the above analogy:
   a. Does making chemical bonds release or require energy? Why? Relate this to stability of the atoms (question #1).

   b. Does breaking chemical bonds release or require energy? Why? Relate this to the stability of the atoms (question #1).
6. A chemical reaction always involves both breaking bonds (of the reactants) and making bonds (of the products). Therefore a chemical reaction may absorb energy overall or release energy overall (compare how much energy is required to break reactants to how much energy is released when products form).

   Endo = within    Exo = outside    therm = heat

Write a definition for the following types of reactions:
   Endothermic
   Exothermic

7. Which type of reaction would involve stronger (more stable) bonds in the products than in the reactants? Which would involve stronger (more stable) bonds in the reactants than in the products? Explain.

8. The following are energy diagrams for endothermic and exothermic reactions. Next to each diagram write out in words what the graph is telling you.

   ![Endothermic Diagram]
   ![Exothermic Diagram]

9. Energy is a driving force of chemical reactions. Look at the diagrams above. Which type of reaction do you think tends to occur on its own (spontaneous) and which type tends to not occur on its own? Explain your answer. Hint: think of the graph like a hill and the chemical reaction as a ball that rolls up or down the hill.

10. The second driving force of chemical reactions is entropy. Define entropy.

11. State the second law of thermodynamics.
12. Explain how each of the following is an example of increasing entropy:
   a. Liquid water being heated until it all evaporates as a gas

   b. Sugar being broken down in cells to release energy to do work (cellular respiration). Hint: The overall chemical equation for this is \( C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \)

   c. A log being burned

13. Give two examples of chemical reactions or processes that living organisms undergo that decreases the entropy of their cells or bodies.

14. The natural direction of change is always toward more entropy. How is it then that organisms are able to become more organized? Are they defying the laws of the universe? Explain your answer.

Now let's put it all together... Gibbs Free Energy

15. Free energy is the energy of a system that is available to do work. Gibbs free energy (\( \Delta G \)) is a measure of the useful energy of a system. Gibbs free energy takes into account the enthalpy (heat) change in a reaction as well as the change in entropy. The changes in enthalpy and entropy are calculated by taking the values of the products—the values of the reactants.

<table>
<thead>
<tr>
<th>Gibbs Free Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta G = \Delta H - T \Delta S )</td>
</tr>
<tr>
<td>( \Delta G = \text{change in Gibbs free energy} )</td>
</tr>
<tr>
<td>( \Delta S = \text{change in entropy} )</td>
</tr>
<tr>
<td>( \Delta H = \text{change in enthalpy (heat)} )</td>
</tr>
<tr>
<td>( \Delta T = \text{absolute temperature (in Kelvin)} )</td>
</tr>
</tbody>
</table>

If \( \Delta H < 0 \) would the reaction be endothermic or exothermic? Explain.

What type of \( \Delta S \) value would represent ice melting (positive, negative, or zero)? Explain.
16. Determine whether each of the following reactions will be spontaneous or nonspontaneous:

\[ \Delta G < 0 \]
\[ \Delta G = 0 \]
\[ \Delta G > 0 \]

17. If \( \Delta G > 0 \) the reaction is endergonic. If \( \Delta G < 0 \) the reaction is exergonic. Define the terms endergonic and exergonic in your own words in terms of free energy and spontaneity.

18. Under what conditions could an endothermic reaction be spontaneous. Explain your answer. (Hint: what other term is included in Gibbs free energy and what would that value have to be in order to offset a positive enthalpy change?).

19. How could a spontaneous reaction be used to drive a nonspontaneous reaction? This coupling of reactions is very important in biological systems! Use the diagram to help you think about it.
AP Biology Lab:
The Breakdown of Hydrogen Peroxide by Catalase

The characteristics of life are the result of chemical reactions occurring within the cell. These chemical reactions take place at unusually high rates because of the participation of a group of protein molecules known as enzymes. While enzymes speed up the rate of the chemical reaction they are not consumed during the reaction. Therefore, each molecule of the enzyme can continuously repeat the reaction it regulates.

According to current theory, the enzyme combines temporarily with a specific substrate molecule forming an enzyme-substrate complex. This union of enzyme and substrate is thought to place added stress on bonds holding the substrate molecule together. So, the energy needed to initiate the bond breaking process is lower and the chemical reaction can take place at a much lower temperature. These assumptions are illustrated in the following reaction.

Enzyme + Substrate → Enzyme-Substrate Complex → Enzyme + Products

Catalase, is a common enzyme that converts hydrogen peroxide (H₂O₂) into water (H₂O) and oxygen (O₂). This reaction is shown in the following equation.

\[
\text{Catalase} + 2\text{H}_2\text{O}_2 \rightarrow \text{Catalase} + 2\text{H}_2\text{O} + \text{O}_2
\]

The rate of the conversion of hydrogen peroxide into water and oxygen is measurable in several different ways. One method measures how rapidly oxygen is released when catalase is added to the hydrogen peroxide. If the reaction occurs in a sealed container the oxygen produced causes an increase in the pressure within the container. This pressure increase is measurable using a special sensor.

The sensor produces an electrical signal that is proportional to the increase in pressure. The signal is digitized by the LabPro interface and then sent to the computer for recording and display. As the measurements are recorded on the screen, a curve representing the pressure change in the reaction vessel is drawn that represents the rate of oxygen release. If the curve is steep the reaction is rapid. If the curve has a slight incline the reaction is slow. A flat line shows there is no reaction.

Use the introductory pages of "Lab Two Enzyme Catalysis" in the AP Biology Lab Manual for additional background on enzymes, factors that affect enzyme activity, catalase, and calculating the initial velocity of a reaction.

Part I of this laboratory demonstrates the existence and qualitative comparison of the concentration of catalase in four living tissues, potato, liver, apple, and hamburger. Part II establishes the standard procedure for gathering quantitative data when measuring the rate of a reaction involving the breakdown of hydrogen peroxide in the presence of catalase. Part III of this experiment will be designed by a team of students to evaluate the effect of several variables on the rate of the breakdown of hydrogen peroxide in the presence of catalase.

Part I: Catalase in Living Tissue

1. To observe the reaction to be studied, transfer 10 ml of 1.5% H₂O₂ into a 50 mL glass beaker and add 1 mL of the stock catalase (400 units/mL) solution. The bubbles coming from the reaction mixture of O₂ which results from the breakdown of H₂O₂ by catalase.

2. To demonstrate the effect of boiling on enzymatic activity, transfer 5 ml of purified catalase to a test tube and place it in boiling water for 5 minutes. Transfer 10 mL of the 1.5% H₂O₂ into a 50 mL glass beaker and add 1 mL of the cooled, boiled catalase solution. How does the reaction compare to the one using the unboiled catalase?

3. Using the same size “cube” of potato, liver, hamburger, and apple, demonstrate the presence of catalase in living tissue by first macerating the tissue with a razor blade on a glass or plastic plate (not on the table top), adding this to a test tube, and adding 5 mL of 1.5% H₂O₂. Note the relative quantity of bubbles formed in each test tube. Try performing the glowing splint test to check for the existence of oxygen gas. The splint may be placed into the test tube or a second test tube can be inverted over the first to catch any released gas. Rank the tissue in order of their catalase concentrations in the box below.

<table>
<thead>
<tr>
<th>Highest</th>
<th></th>
<th>Lowest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Part II: Standard Procedure for Measuring the Rate of the Breakdown of H₂O₂ with Catalase

Vernier Equipment
Pressure sensor, LabPro interface, gas pressure accessories kit as seen in the above photo.

Materials
Safety goggles, 50 ml beaker, 1.5% hydrogen peroxide at pH 7, catalase solution (400 units/ml) at pH 7, 1 ml syringe, 10 ml syringe, 125 ml beaker.

Safety
Wear safety goggles at all times during this investigation.

Procedure

1. Place 10 ml of 1.5% hydrogen peroxide solution buffered at pH 7 into the 125 mL reaction flask using a 10 ml syringe that is to be used only for that purpose.

2. Add 1 ml of 400 units/ml catalase solution at pH 7 (to be kept on ice at all times) to the silver enzyme vessel (thimble) using a 1 ml syringe that is to be used only for that purpose.

3. Place the enzyme vessel inside the reaction flask without spilling the enzyme.

4. Insert the two hole stopper into the reaction flask with a twisting motion.

5. Seal the system by closing the valve. It should be turned at a right angle to the tube. Click on collect. Immediately swirl the flask to tip the enzyme vessel. This step spills the enzyme into the outer reaction flask. Continue swirling the flask gently as long as data is being collected.

6. The line plotted on the graph should rise sharply. Stop the data collection when the sample number reaches 200. Open the valve to release the pressure. Print the graph. From this graph, calculate the initial velocity of the reaction.

7. Remove the stopper from the reaction flask. Dump the contents of the flask. Wash the reaction flask and the enzyme vessel thoroughly.
8. Repeat this exact same procedure three times. Calculate the initial velocity for each trial. They should be nearly the same if your procedure is accurate.

**Part III: Experimental Design to Answer Two Questions**

Your team will be given **two** of the following questions to evaluate. You must:

- design your own procedure to evaluate the questions using the equipment and solutions available in our lab; you may need to make some dilutions of solutions in order to run your trials
- perform enough trials to adequately evaluate the questions; repeat trials as needed
- establish a control for your experiment
- hold all variables except the experimental one constant
- print out all graphs from your experiment and save them for your report
- keep careful notes of your experiment so you can write a detailed description of your procedure and construct appropriate data tables

The questions to ponder:

1. What is the effect of increasing catalase concentration on the rate of the breakdown of $H_2O_2$?
2. What is the effect of increasing hydrogen peroxide concentration (the substrate) on the rate of the breakdown of $H_2O_2$ in the presence of catalase?
3. What is the effect of increasing pH on rate of the breakdown of $H_2O_2$ in the presence of catalase? What is the optimal pH at which catalase functions?
4. What is the effect of increasing salt concentration on rate of the breakdown of $H_2O_2$ in the presence of catalase? What is the optimal salt concentration at which catalase functions?
5. What is the effect of increasing temperature on the rate of the breakdown of $H_2O_2$ in the presence of catalase? What is the optimal temperature at which catalase functions?

You will be using the mini-poster format to report your results.
**INTRODUCTION**

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>BACKGROUND</th>
<th>HYPOTHESIS</th>
</tr>
</thead>
</table>

**METHODODOLOGY**

<table>
<thead>
<tr>
<th>PROCEDURE</th>
<th>MATERIALS</th>
<th>STATISTICAL TESTS</th>
</tr>
</thead>
</table>

Include your own picture(s)

**RESULTS**

The Effect of Temperature on Catalase Reaction Rate with Hydrogen Peroxide

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Reaction Rate (M/hr/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>0.23</td>
</tr>
<tr>
<td>30</td>
<td>0.37</td>
</tr>
<tr>
<td>40</td>
<td>1.12</td>
</tr>
<tr>
<td>50</td>
<td>0.91</td>
</tr>
<tr>
<td>60</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The Effect of Temperature on the Rate Catalase Breaks Down Hydrogen Peroxide

**DISCUSSION**

**LITERATURE CITED**
AP Lab #13: Peroxidase: Setting up the Spectrophotometer

INTRODUCTION
A spectrophotometer is a valuable instrument in biology. It allows us to measure and compare the concentration of materials in solution with relative ease. It is therefore important for you to understand how the spectrophotometer works so that you understand the data that you collect from it.

WHAT DOES A SPECTROPHOTOMETER DO?
A spectrophotometer is an instrument that measures either the transmittance of light through a solution or the absorbance of light by a solution.

Light can either pass through an object or be stopped by an object which is in its path. We can easily see this if we try looking at a light through a piece of clear plastic — all of it is transmitted through and none of it is absorbed. Then try to see the same light through a piece of cardboard — all of it is absorbed by the cardboard and none of it is transmitted through.

In this way, you can now see that transmittance refers to the light which passes through a solution. Absorbance, on the other hand, refers to the light which cannot pass through a solution, but rather is absorbed by the solution.

For example, if we pass a beam of white light through a blue glass, not all the light passes through the glass to the other side — some of it is absorbed by the glass. The light which does pass through the glass is said to be transmitted and its measure is called transmittance. So, if your beam of white light is 40% absorbed by the glass, it can also be said that the light is 60% transmitted.

It is now clear (pardon the pun) that the darker or denser a substance is the more light it will absorb and theses light it will transmit.

WHAT ARE THE SCALES ON A SPECTROPHOTOMETER?

Transmittance
Transmittance is an arithmetic scale that is divided into one hundred equal parts, so transmittance can be expressed as a percent. A value of 100% transmittance means that all the light has passed through the material in the cuvette and none was absorbed. A value of 0% transmittance means that no light passed through the sample in the cuvette.

Absorbance
Absorbance is a logarithmic scale with unequal divisions. A value of 0.0 absorbance means that no light has been absorbed by the material and all the light has been transmitted. A reading all the way at the other end of the scale means that all the light has been absorbed by the material.

WHAT IS THE “BLANK” AND WHY DO WE HAVE TO USE IT?
All substances, clear or not, absorb some light. Therefore, if we wish to measure the absorbance (or the transmittance) of a material like protein in solution, we must not only consider the light absorbed by the protein, but also the light absorbed by the cuvette and the light absorbed by the solution in which the protein is dissolved.

In order to measure only the absorbance of the protein in the solution, we must “subtract” out the absorbance due to the cuvette and the solvent in which the protein is dissolved.

If our protein is dissolved in water, and we are trying to assess how much protein is in solution, we would ultimately want to measure how much light is absorbed by the protein alone and not the how much light is absorbed by either the water or the cuvette. In order to subtract out these factors, we prepare another cuvette with only water in it. This is called the “blank.” In general, the blank is a cuvette which contains everything that is in the sample (or experimental) cuvette, except the one material whose
absorbance we are measuring.

To use the blank, you could measure your experimental cuvette, then measure your blank cuvette and find the difference between them. However the spectrophotometer has a built in calibration system that allows you to avoid this calculation and its much like zeroing an electronic scale. Follow this procedure:

**To Calibrate the SpectroVis:**

1. Connect the SpectroVis to the USB port.
2. Open LoggerPro
3. Allow the SpectroVis to complete its warm-up.
4. Choose Calibrate → Spectrometer from the Experiment Menu.
5. Fill a cuvette about ¾ full with distilled water* and place in the cuvette holder.
6. Follow the instructions in the dialog box to complete the calibration and then click “OK.”
7. Take the blank out and now you are ready to read the absorbance of the material in solution without having your data affected by the absorbance of the cuvette or the solvent.
8. Also be aware that fingerprints on the cuvettes can affect your readings, so all cuvettes need to be wiped clean with a lens paper before inserting them into the spectrophotometer.

*The cuvette to use for calibration should actually contain all the materials except he one for which you are measuring the absorbance. For the Peroxidase experiment, this would include the water, buffer, hydrogen peroxide, and enzyme. It would NOT contain the guaiacol.

**WHAT WAVELENGTH OF LIGHT DO I USE?**

Most of the material we see around due is colored due to light reflection. Why do we see a blue sky? White light hits the layers of the atmosphere and all the colors of the spectrum are absorbed, except the color blue, which is reflected back to our eyes. Why is your shirt red? Because white light hits the shirt fabric and all the colors of the spectrum are absorbed, except the color red, which is reflected back to our eyes.

Everything around us absorbs certain colors (wavelengths of light) better than other colors. Since the spectrophotometer measures absorbance of light it would work the best in an experiment when we use the color light that is best absorbed by the material we are examining. In other words, the spectrophotometer shines specific wavelengths of light through the cuvette. We will get more accurate readings in our experiment, if we set the spectrophotometer to shine through the cuvette the wavelength of light that the material in the cuvette absorbs the most.

How do we know which color (wavelength) of light is absorbed best by the material we are testing for in the cuvettes? To answer that question, we have to complete a test called an “Absorption Spectrum” on the material.

How do we run an “Absorption Spectrum”? Just measure the absorbance of the material over a range of wavelengths. Then plot a graph of absorbance vs. wavelength of light and we are likely to get a curve which looks like the one to the right. We may do this for our experiment.

For the peroxidase experiment, previous work shows the absorbance of guaiacol to be maximal at 470 millimicrons.
To Set the SpectroVis to a wavelength of 470 nm:

1. Choose Experiment → Set up Sensors → Spectrophotometer 1
2. This will open this dialog box:

3. Change the Samples to Average to 5 (this will average 5 readings at once)

4. Change Wavelength Range to: 465 - 475
   This will cause the SpectroVis to measure the absorbance at a wavelength of 470 within a range of 5 on either side of 470.

5. Now click on the rainbow graph in the upper right hand corner of the dialog box.

6. Choose Configure Collection

7. Set Collection Mode → Choose Abs vs Time

8. On the right, check the wavelength 472 (or as close as you can get) and make sure no other wavelengths are also chosen then “Ok.”

9. Close the dialog box. The SpectroVis is now ready to read the absorbance. It will read for 200 seconds and then it will need to be started over.

10. Read the absorbance from the table.
AP Biology Digestion & Nutrition Diagrams

1. Pepsinogen and HCl are secreted.
2. HCl converts pepsinogen to pepsin.
3. Pepsin activates more pepsinogen.

Carbohydrate digestion

- Oral cavity, pharynx, esophagus
  - Salivary amylase
  - Smaller polysaccharides, maltose

- Stomach
  - Polysaccharides
  - Disaccharides (starch, glycogen) (lactose, maltose)
  - Mucosa and other disaccharides

- Lumen of small intestine
  - Pancreatic amylase
  - Malto and other disaccharides

- Epithelium of small intestine (brush border)
  - Disaccharides
  - Monosaccharides

Protein digestion

- Proteins
- Small polypeptides
- Peptides
- Amino acids
- Peptideases, carboxypeptidase, and aminopeptidase

Nucleic acid digestion

- DNA, RNA
- Nucleotides
- Nucleosides
- Nucleosides and phosphates
- Nitrogenous bases, sugars, phosphates

Fat digestion

- Fat globules
- Bile salts
- Fat droplets
- Glycerol, fatty acids, monoglycerides
Major Human Digestive Organs

1. 
2. 
3. 
4. 
5. 
6. 
7. 
8. 
9. 
10. 
11. 
12. 
13. 
14.
AP Bio LAB: Computer Analysis of Diet

STEP ONE
1. Go to the FitDay web site at http://www.ﬁtday.com/
2. Choose the orange button “Get Your Free Account”
3. Create an account with a username and password (in case you need to come back to this info); don’t give it your real email or you may get lots of junk email; type in the personal info (sex, birthday, height, weight, activity level) accurately

STEP TWO
4. Search for each of the foods on your food list. If you can’t find your exact food, browse and find a food that comes close.
5. Adjust the servings and serving size to match as closely as possible your intake.
6. Continue to input foods until all your foods that are in your list have been added.
7. You may go back and modify your food log in case you make any mistakes.

STEP THREE
8. From the top menu bar, choose log → activities.
9. Input your activities for the past 24 hours.

STEP FOUR
10. Return to the food log page. On the right you will see a graph with a drop down menu. Look at the following graphs about your food totals and calories burned
1. Calorie Balance – How does the calories eaten compare to the calories burned?
2. Calorie Intake: From which food group do you get most of your calories?
3. Record your percentages:
   - Fat
   - Carbs
   - Protein
4. Calorie Breakdown – Look at the calories from fats. Compare your calories from the three fat categories:
   - Saturated fats
   - Polyunsaturated fats
   - Monounsaturated fats
11. Look at your fiber. How many grams of fiber are you consuming.
   a. Now from the top menu bar, choose reports
   b. Obtain and print the following reports for you:
      - Nutrition – as a graph
      - Nutrition – as a table (see the lower right corner)
      - For which vitamins and minerals are you deficient? What problems could you have if this was chronic?
      - For which vitamins and minerals are you in excess?

STEP FIVE
Use your personal results, your textbook, and any other resources to answer the following questions.

1. Compare your calorie intake to the calorie intake you should intake to match your energy expenditure. If you ate like this daily, would be you either undernourished or overnourished? Explain. In what category do you spend most of your calories?

2. Using the resources available, compare your fat, fiber, protein and carbohydrate intake as a percent of your calories to that suggested for a person your age and sex. Where do you fall outside acceptable ranges? If you ate this way everyday, would you be considered malnourished? Explain. (Note: Adults should eat 25 - 30 grams of fiber per day.)
3. Which is higher, your saturated fat intake or your unsaturated fat intake? Explain which is healthier. Are less than 25% of your calories from sources of fat?

4. Using your report on nutrient requirements, evaluate your intake of vitamins and minerals. For which are you deficient and which are you in excess? What could you be susceptible to due to your deficiencies and excesses?

5. Adjust your original diet so that it falls within all the proper dietary guidelines. What foods could you add and/or subtract to obtain a healthier diet? Print out this food list.

   Revise your food list so that it contains
   • within 95-105% of your calorie and protein needs;
   • at least 100% for your fiber needs;
   • less than 25% of your calories from fat;
   • at least 100% for each of the vitamins and minerals listed.

6. Complete the table below as explained by your teacher.
Introduction & Background Information:

**Heterotrophs:** Animals, some bacteria, fungi and some protists are heterotrophs, dependent on a regular supply of food derived from other organisms. Animals exhibit a great variety of nutritional adaptations. For example, a snowshoe hare is adapted for life in northern forests. Able to obtain all their nutritional needs from plants alone, hares and rabbits have a large intestinal pouch housing prokaryotes and protists that digest cellulose. When deep snow covers the ground, a hare can live on pine, fir and spruce branches. Most animals eat other organisms, dead or alive, whole or by the piece. Exceptions to this include parasitic animals, such as tapeworms, which absorb organic molecules across their body surface. In general, animals fit into one of three dietary categories. **Herbivores,** including gorillas, cows, hares and many snails, eat mainly autotrophs (plants and algae). **Carnivores,** such as sharks, hawks, spiders and snakes, eat other animals. **Omnivores** regularly consume animals as well as plant or algal matter. Omnivores animals include cockroaches, crows, raccoons and humans.

**Ingestion:** Animals ingest (take in) their food in a variety of ways. Many aquatic animals are **suspension-feeders** or **filter-feeders** that sift small food particles from the water. Clams and oysters, for example, use their gills to trap tiny morsels, which are then swept along with a file of mucus to the mouth by beating cilia. Baleen whales, the largest animals to ever live, are also suspension feeders. They swim with their mouths agape, straining millions of small animals from huge volumes of water forced through screen-like plates attached to their jaws. Sponges are sessile, primitive animals who are filter feeders. Watch a sponge feeding here (5 minutes): http://www.youtube.com/watch?v=RmPM965-1c&NR=1

**Substrate-feeders** live in or on their food source, eating their way through the food. Examples are leaf-miners, which are larvae of various insects that tunnel through the interior of leaves. You can see one eating inside a leaf here: http://www.youtube.com/watch?v=PP8l-T39n3E

Earthworms are substrate-feeders, too, eating their way through the dirt, salvaging partially decayed organic material consumed along with the soil. **Fluid-feeders** make their living by sucking nutrient-rich fluids from a living host. Mosquitoes and leeches suck blood from animals and since they cause harm are considered parasites. Hummingbirds and bees, on the other hand, benefit their host plants, transferring pollen as they move from flower to flower to obtain nectar. Most animals are **bulk-feeders** that eat relatively large pieces of food. Their adaptations include tentacles, pincers, claws, poisonous fangs, and jaws and teeth that kill their prey or tear off pieces of meat or vegetation.

**Digestion:** Once food is ingested, it must then be digested, hydrolysing the food’s macromolecules into monomers that the body can use. The most significant monomers are amino acids, monosaccharides, glyceral and fatty acids. Digestion can be either **intracellular,** that is, in vacuoles within cells, or **extracellular,** in a digestive cavity in an animal’s body. Most multicellular organisms like animals have extracellular digestion that first involves a mechanical process where large food chunks are physically broken down into small pieces by teeth followed by chemical digestion where enzymes break the large macromolecules into smaller building blocks by hydrolysis for easier absorption through the intestinal cells. Most unicellular organisms like protists utilize intracellular digestion where small food pieces are first ingested by phagocytosis into a food vacuole, which then combines with a lysosome and its hydrolytic enzymes in order to complete digestion. Some animals live symbiotically with protists. Termites, for example, are bulk feeders, ingesting small chunks of wood. However, they lack enzymes capable of hydrolyzing cellulose into simple sugars. Protists, such as *Trichonympha,* inhabit the intestine of termites where they digest the cellulose in the wood ingested by the termites. The termites then assimilate the monomers (simple sugars) and are able to survive on their diet of wood.

**Purpose:** The purpose of this lab activity is to observe and describe the ingestion of several organisms, specifically *Paramecium caudatum,* hydra, and planaria and to find the intestinal symbionts of termites.

**Materials:**

<table>
<thead>
<tr>
<th>living hydra culture</th>
<th>Daphnia culture (pond?)</th>
<th>extra aquarium water</th>
</tr>
</thead>
<tbody>
<tr>
<td>living planaria culture</td>
<td>droppers</td>
<td>microscope slides</td>
</tr>
<tr>
<td>living <em>Paramecium</em> culture</td>
<td>liver</td>
<td>glass coverslips</td>
</tr>
<tr>
<td>Yeast stained with Congo Red</td>
<td>watch glass</td>
<td>saline solution</td>
</tr>
<tr>
<td>Methyl cellulose (3%)</td>
<td>depression slides</td>
<td></td>
</tr>
</tbody>
</table>
Procedures:

A. Paramecia
   Option: Watch DVD program on the feeding of paramecium

1. *Paramecia* are microscopic protists that move quickly using their cilia. They can be slowed by using a ring of methyl cellulose or some cotton fibers. Place a ring methyl cellulose or cotton fibers on a clean microscope slide.
2. Place a small drop of Paramecia culture in the center of the ring or on the cotton fibers.
3. Add a small drop of yeast suspension stained with Congo red to the Paramecia drop. (NOTE: Congo red is a pH indicator that is red at or above pH 5 and is various shades of blue at a pH of less than 5.
4. Cover with a clean coverslip and immediately observe what happens with a microscope.
5. Locate one Paramecium and observe what happens to the to the stained yeast cells. The Paramecia usually ingest some of the yeast cells very quickly, perhaps within 10 seconds of adding the yeast.
6. Study the food vacuole formed inside a Paramecium and observe it for at least 10 minutes. HINT: You will need to continuously focus up and down “through” the Paramecium and regulate the light carefully.
7. Record the color of the ingested yeast in a data table you construct every two minutes for a total of 10 minutes.
8. On the outline of a paramecium below, show the path of the ingested yeast using arrows, and add and/or label the macronucleus, micronucleus, cell membrane, cytoplasm, oral groove, food vacuole forming, food vacuole in the cell, contractile vacuole, anal pore, and cilia to the sketch. (You may need to research outside of lab to be able to finish this sketch.)

9. (NOTE: If your Paramecium does not eat, a short video clip is available)
B. Hydra

1. Hydra are fragile animals. Treat them gently.
2. Obtain a watch glass. Fill the watch glass about half way with pond water.
3. Your teacher will place one or two hydra along with several drops of culture water into the watch glass.
4. Place the watch glass on the stage of your stereo microscope. Observe your hydra. Make a sketch in the box on the next page of the hydra, labeling mouth, tentacles, nematocysts (bulges on the tentacles), basal disk (end opposite to the tentacles) and gastrovascular cavity. If asexual buds are be visible on your hydra, draw and label these as well.

Hydra Sketch:

5. Touch the hydra very gently with the end of a wooden handled probe. Note its response.
6. As you observe your hydra under the scope, your teacher will deliver a small dropper of Daphnia and place them on top of your hydra. Observe the behavior of the hydra from the time it makes contact with a Daphnia until it has completely ingested the Daphnia. You may be lucky enough to observe egestion (removal of undigested material from the hydra) as well.
7. Describe your observations here:

Observations of Hydra Ingestion

8. (NOTE: If your Hydra does not eat, a short video clip is available)
C. Planaria

1. Obtain one or two planaria in a watch glass half full with aquarium water.
2. Observe the planarian under the dissecting microscope.
3. It's pharynx may not yet be visible. Note other features of the planarian: head, eyespots, auricles (where ears would be), bilateral symmetry, pharynx and intestines.
4. Place a small piece of rinsed, fresh liver in water with the planarian.
5. Watch, looking for extension of the pharynx. This can take a while. Be patient.
6. Set the planarian aside and continue to observe periodically for ingestion activities while you are completing the rest of the lab.
7. (EXTENSION: Your teacher may suggest you use your planarian for a study in regeneration.
Planarian are quite remarkable in their ability to reform parts of their body regardless of which part of the worm has been cut. Place your planarian on an ice cube or very cold piece of glass. Using a sharp razor blade, make one cut of your one choosing. Place the pieces in separate Petri dishes partially filled with clean pond water and cover to reduce evaporation. Place them in a cool place worth subdued light. Do not feed. Observe over he next several days. Looking for regeneration.)
8. (NOTE: If your Planarian does not eat, a short video clip is available)

D. Termites & Symbiotic Protists

1. Obtain a microscope slide and coverslip.
2. Place a drop of saline solution in the center of the microscope slide.
3. With a forceps, gently grasp a termite by the thorax. Be careful not to allow any termites to escape!
4. Lightly squeeze the thorax of the termite. This will cause the termite to evacuate its intestine, producing a milky fluid.
5. Dip the termite's abdomen in the drop of saline applied to the microscope slide.
6. Gently cover the drop of saline with a coverslip, being careful not to trap any air bubbles underneath.
7. Observe the prepared mount under low and then high power.
8. (Alternate Procedure: Using two wooden handled needles place one on the front end of the termite and one on the abdomen. When these needles are pulled in opposite directions, the alimentary canal will open and reveal the wealth of protists within. A drop of distilled water should be added to the slide and a coverslip place in a normal wet mount fashion. This will allow the protists to swim from the alimentary canal and allow the students much easier viewing.)
9. Draw the shape of the intracellular symbionts in the box on the next page and label them using the chart on the last page of this lab.
E. Ingestion in Carnivorous Plants

Watch segments of Death Trap illustrating ingestion by the venus fly trap and other carnivorous plants.

Alternates:
Life - Venus Flytraps: Jaws of Death - BBC One: 
http://www.youtube.com/watch?v=QZeK5f0LmY

Venus fly trap - The Private Life of Plants - David Attenborough - BBC wildlife: 
http://www.youtube.com/watch?v=kUGVtKdgwo

How flesh-eating pitcher plants trap insects: http://www.youtube.com/watch?v=ya2ndp1OrPQ

Midge on Drosera capensis (Sundew): 
http://www.youtube.com/watch?v=cZ7Fws1HaL0&feature=related

Follow-up Questions

Living organisms can be tricky to observe and they do not always cooperate! Answers to the following questions come from not only your observations but from your textbook, from video clips, and from web searching.

Paramecium
1. Identify the Paramecium’s Kingdom: ________________________________

2. The Paramecium uses (intracellular or extracellular) digestion.

3. Read about how an amoeba captures it’s prey or watch it at http://www.youtube.com/watch?v=W6rniMxtKU. Identify one similarity and one difference between how an amoeba and a paramecium catches its prey.
**Hydra**

4. Identify the Hydra's Kingdom: ___________________________ and Phylum ___________________________.

5. The Hydra uses (intracellular or extracellular) digestion.

6. How many body openings (one or two) does the hydra have? ("Body openings" are referring to a mouth and an anus.)

7. What are nematocysts? How do they aid the hydra in prey capture?

8. Describe how the hydra removes undigested food.

**Planarian**

9. Identify the Planarian's Kingdom: ___________________________ and Phylum ___________________________.

10. The Planarian uses (intracellular or extracellular) digestion.

11. How many body openings (one or two) does the planarian have?

12. Explain why the intestine is branched in the planarian.

**Termites**

13. Identify the Termites's Kingdom: ___________________________ and Phylum ___________________________ and Class ___________________________.

14. The Termite uses (intracellular or extracellular) digestion.

15. How many body openings (one or two) the termite have? Explain how this is considered an advantage compared to the hydra and planarian.

16. The termite and it's protists have a **mutualistic** relationship. What does mutualism mean in this context? Identify what the termite does for the protists and what the protists do for the termites.

17. Newly hatched termites must ingest the hindgut fluids from other termites in order to survive. Why is this the case?
Reference for Common Intestinal Symbionts of Termites

Trichonympha

Trichomitopsis

Trichomonas

Streblomastix

Hexamastix

Teacher Notes Jan. 2011