BIOLOGY 12 **NAME: _____**

ENZYMES

BACKGROUND INFORMATION:

Energy: is defined as the ability to do __work__ or bring about change. A living organism must constantly perform work in order to maintain its organization, to grow, and to reproduce.

Two Laws of Thermodynamics:

Thermodynamics is the study of energy relationships and exchanges.

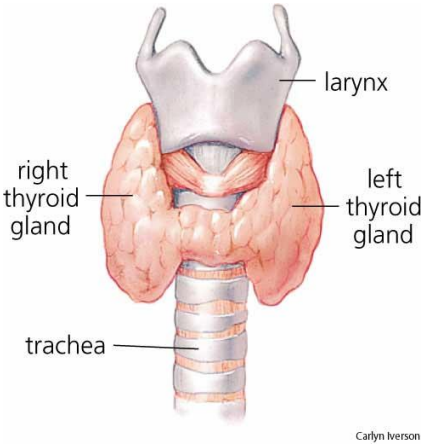
- First Law of Thermodynamics: states that energy can neither be __created__ nor __destroyed__.
- Second Law of Thermodynamics: One usable form of energy cannot be completely converted into another usable form.

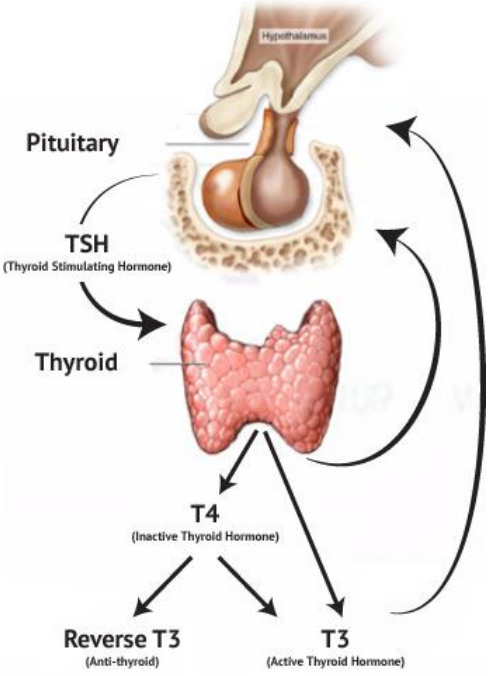
METABOLISM:

Cells are not static; they are dynamic. Microscopic observations of cells give us the impression that cells are inactive; actually, cells are constantly active. Pinocytotic and phagocytotic vesicles are constantly being formed, organelles are moving about, and cell division may be taking place. A vital part of this activity is constantly occurring __chemical reactions__, which collectively are termed the __metabolism__ of the cell.

a) **Metabolism Supplement:**

- THYROXIN (hormone) SPEEDS METABOLIC RATE





The **thyroid gland** is a large gland located in the neck, where it is attached to the trachea just below the larynx. It consists of right and left lobes which are connected.

It is here that **thyroxin** (thyroid hormone) is produced under stimulation by thyroid stimulating hormone (TSH) from the anterior lobe of the pituitary gland (brain).

- **Thyroxin increases the metabolic rate.**
  - It does not have one target organ; instead, it stimulates all cells of the body to metabolize at a faster rate.
  - More glucose is broken down and more energy is utilized.

Excessive thyroxin secretion results in signs of an increased metabolism = Hyperthyroidism.
Symptoms: weight loss, extreme nervousness, raised body temperature, profuse sweating, high blood pressure, higher pulse rate, and protruding eyeballs because of edema (fluid in the tissues) in eye socket tissues.

Inadequate thyroxin secretion results in signs of slowed metabolism = Hypothyroidism.
Symptoms: weight gain, lethargy, loss of hair, lowered body temperature, low blood pressure, slower pulse rate, and thickness and puffiness of the skin (myxedema).

b) Chemical Reaction Rates:

Molecules are in constant motion. Although chemical reactions sometimes occur when molecules collide, most reactions do not occur _spontaneously_ unless they are activated in some way. The rate at which a chemical reaction proceeds often depends on the _temperature_. Note: Recall the Kinetic Molecular Theory: As heat is added to a substance, its particles gain kinetic energy which causes them to move _faster_. In a chemical reaction, this results in greater _collisions_ between reactant molecules, and a greater chance of them reacting with each other.

- **The kinetic energy required to cause molecules to react with one another and start a chemical reaction is called the energy of activation or _Activation Energy (E_a)_.**

The requirement for activation energy applies to all types of reactions (exothermic and endothermic). Although a chemical reaction may have the potential for producing energy, it will not proceed unless the reactant (starting) molecules possess the necessary energy. Therefore, whether a reaction gives off energy or absorbs it, **some energy must be added initially to start the reaction (activate it!).**

- Imagine a rock on top of a hill. The rock has potential energy that can be converted into kinetic energy as it rolls down the hill. But to start the rock rolling, someone must push or kick it. The energy it takes to start the rock rolling is similar to activation energy.

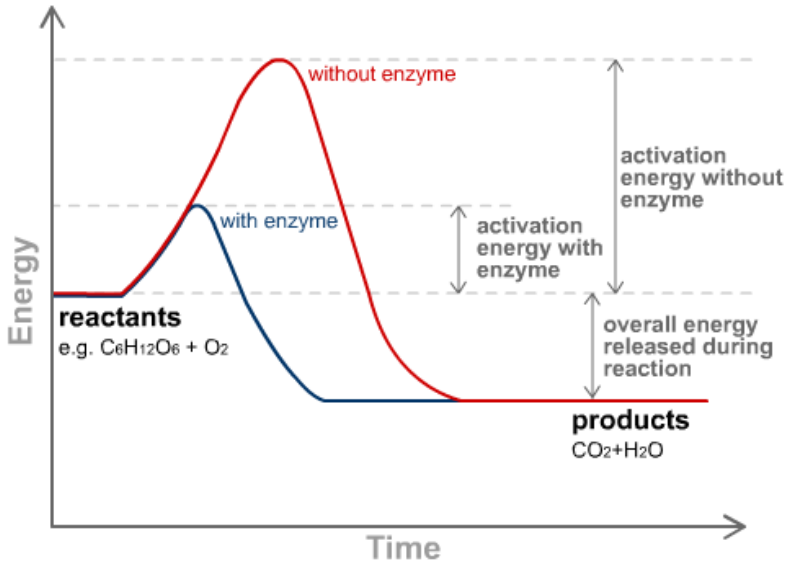
ENZYMES: CATALYSTS IN CELLS

Adding activation energy in the form of heat to increase the number of collisions and thus the probability of a reaction taking place is dangerous – thermal energy could destroy the cells. Chemical reactions must therefore proceed at relatively low temperatures with cells. _Catalysts_ are chemicals that control the speed of chemical reactions without altering the products formed by the reaction. This means that the catalyst remains unchanged after the chemical reaction, and can be used over and over again. Reactions that occur within living organisms are regulated by protein catalysts called _enzymes_.

- ***ENZYME*** = *A protein molecule that functions as an organic catalyst allowing biological reactions to occur faster and at the relatively low temperature of cells.*
- Nearly every chemical reaction that occurs in an organism is made possible by a specific enzyme.
- Without enzymes, many metabolic reactions would occur too slowly to sustain life.

a) How do Enzymes Work?

= _enzymes lower the Activation Energy_
In other words, an enzyme lowers the amount of energy (heat) needed to begin a reaction.



Enzymes have four features in common:

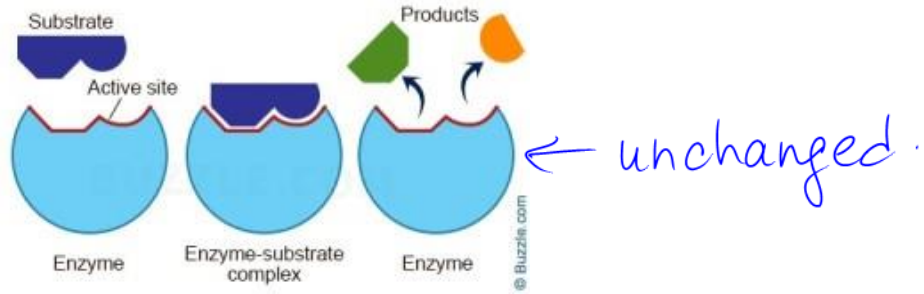
1. An enzyme will not make anything happen that could not happen on its own. But it will make it happen much faster.
2. An enzyme accelerates the rate of reactions – it does not change the outcome of a reaction.
3. In performing its function an enzyme undergoes no net chemical change; i.e. It is the same molecule after it accelerates a reaction as it was before, and can therefore be used over and over again. Therefore, only a small amount of enzyme is actually found in a cell.
4. Each type of enzyme is highly selective or ___specific___ to the reactants it catalyzes.

b) Enzyme-Substrate Complex

Enzymes speed up chemical reactions by lowering the activation energy. They do this by forming an enzyme-substrate complex.

Compounds upon which an enzyme acts are known as ___substrates (reactants)___, and the compounds produced are called ___products___.

- ***SUBSTRATES*** *= the reactants in an enzyme-catalyzed chemical reaction.*



Enzymes lower the amount of activation energy necessary for a reaction to take place by placing its substrates on a precise collision course.

An enzyme is **very specific** in its action and can speed up only one particular reaction or one type of reaction, therefore, many enzymes are often named for their substrates.

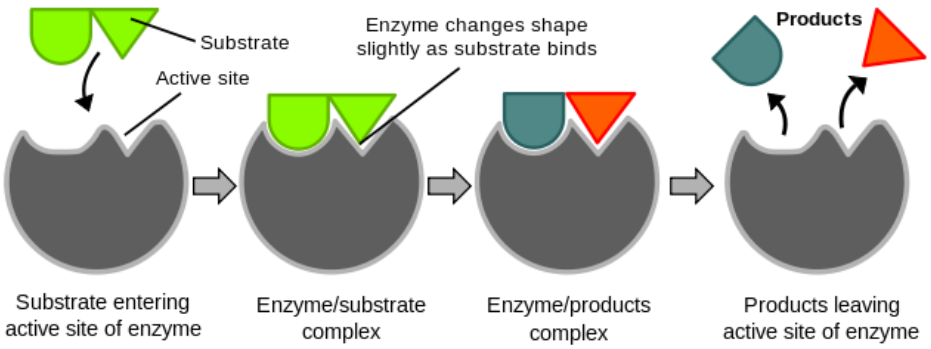
- The name of an enzyme is often formed by adding "___ase___" to the name of the substrate:

i.e.	Substrate:	Enzyme:
	Lipid	___lipase___
	Urea	___urease___
	Lactose	___lactase___

- Some enzymes are also named for the action they perform; for example, dehydrogenase is an enzyme that removes hydrogen atoms from its substrate.

➢ ***ACTIVE SITES*** = A region on the surface of an enzyme where the substrate(s) bind in such a way that they are positioned to react.
In most instances, only one small part of the enzyme called the active site, complexes or binds with the substrate.

➢ ***INDUCED-FIT MODEL OF ENZYME ACTIVITY***
The shape of an enzyme allows an enzyme-substrate complex to form, which explains the specificity of an enzyme. *Note: Recall the folding of the amino acid chain upon itself to produce the uniquely shaped tertiary structure of a protein.* The substrates are seemingly specific to the enzyme because their shapes fit together as a key fits a lock. Current knowledge of enzyme activity has shown that the active site of an enzyme actually undergoes a slight change in shape in order to accommodate the substrates more perfectly. This is called the **induced-fit model** because as binding occurs, the active site is induced (undergoes a slight alteration) to achieve the best fit. After the reaction is complete, the product is released and the active site returns to its original state.



c) <u>The Product: Determined by the Reaction</u>
An enzymatic reaction can bring about synthesis or degradation.

Synthetic reaction:
= The enzyme catalyzes a reaction by holding two substrates in positions in which they can react with each other to form a larger molecule. i.e. 2 amino acids can be joined to form a dipeptide

Degradation reaction:
= The enzyme catalyzes a reaction by twisting a substrate molecule slightly so that a chemical bond is weakened and broken, breaking down the substrate into smaller molecules.

d) <u>Cofactors: Helpers of Enzymes</u>
Many enzymes require a nonprotein molecule or ion to function properly as a catalyst. These necessary nonprotein molecules are called **cofactors.**

- A cofactor is an essential part of the active site. Without the cofactor, the substrate either does not bind to the enzyme at all, or if it does bind, the reaction still does not proceed at any significant rate.
- Many cofactors are ions (inorganic) or coenzymes (organic)

<u>Ions as Cofactors:</u>
Examples: Magnesium ions (Mg^{2+}), Potassium ions (K^{+}), Calcium ions (Ca^{2+}); these ions help to hold certain folds in the tertiary structure of an enzyme in the correct shape to form the active site.

Coenzymes and Vitamins:

Some other cofactors, called coenzymes, are nonprotein organic molecules that bind to enzymes and serve as carriers for chemical groups or electrons. It is a large molecule that the body is incapable of synthesizing without the ingestion of a vitamin.

Vitamins are organic dietary requirements needed in small amounts only. The vitamin becomes part of the coenzyme's molecular structure.

These vitamins are necessary for the formation of the coenzymes listed:

Vitamin	Coenzyme
Niacin	NAD+
B2 (riboflavin)	FAD
Folic Acid	coenzyme in nucleic acid and amino acid metabolism

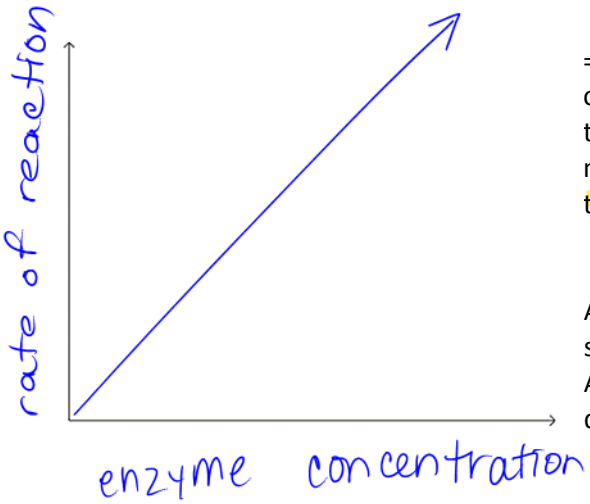
Humans and other animals cannot synthesize vitamins themselves and must obtain them from their diets. A deficiency of any one of these vitamins results in a lack of the coenzyme and therefore a lack of certain enzymatic actions. In humans, this eventually results in vitamin-deficiency symptoms:

i.e. Niacin deficiency – results in a skin disease called pellagra
Riboflavin deficiency – results in cracks at the corner of the mouth
Folic acid deficiency – anemia

e) Factors that Affect Enzyme Activity

It is estimated that a single enzyme can catalyze up to 30 million reactions every minute. Why do some reactions occur much faster than others?
In order to compare reaction rates, examine the different factors that affect enzymes:

1. ENZYME CONCENTRATION

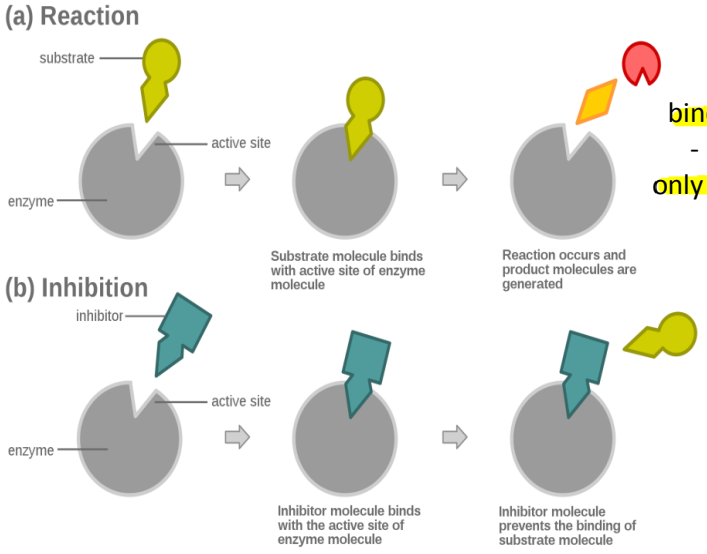


= Assuming that there is plenty of substrate available, increasing the amount of enzyme present will increase the rate of reaction. This occurs because there are more collisions between enzymes and the substrate molecules. As more substrate molecules fill active sites, more products result per unit time.

A cell can increase the amount of enzyme present by increasing protein synthesis of that enzyme.
A cell can also decrease the enzymes available to a chemical reaction by deactivating the enzyme using enzyme inhibitors.

2. Competitive Inhibitors:

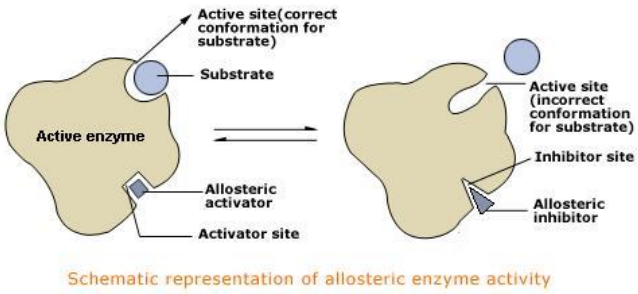
= another molecule that is so close in shape to the enzyme's normal substrate that it competes for the active site on the enzyme



- The competitive inhibitor prevents the substrate from binding either permanently or temporarily
- Inhibiting molecule prevents the chemical reaction because only the binding of the true substrate results in a product

Non-Competitive Inhibitors:

= a molecule that disables the enzyme without competing with the substrate molecule for the active site.



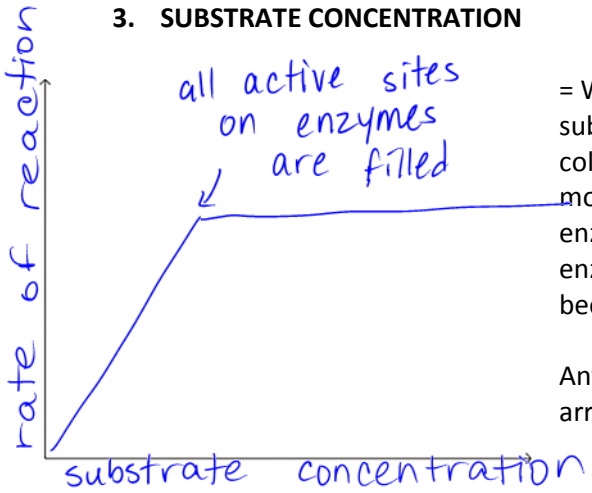
Schematic representation of allosteric enzyme activity

-Non-competitive inhibitor binds to an alternate site on the enzyme, called the allosteric site, which inactivates the enzyme.

-The active site of the enzyme cannot be used while the non-competitive inhibitor is filling the allosteric site.

-This type of inhibition is non-competitive, because the inhibitor does not bind to the active site of the enzyme

3. SUBSTRATE CONCENTRATION

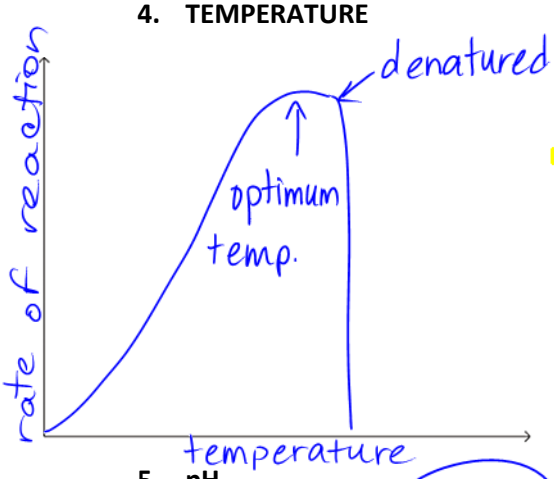


= With fixed amounts of enzyme present, enzyme activity increases as substrate concentration increases. This occurs because there are more collisions between substrate molecules and the enzyme. As more substrate molecules fill active sites, more products result per unit time. When all enzyme's active sites are filled, almost continuously with substrate, the enzyme's rate of activity cannot increase anymore and maximum rate has been reached (plateau).

Any factor that influences the shape of the protein molecule, and hence the arrangement of the active site, will alter the activity of the enzyme.

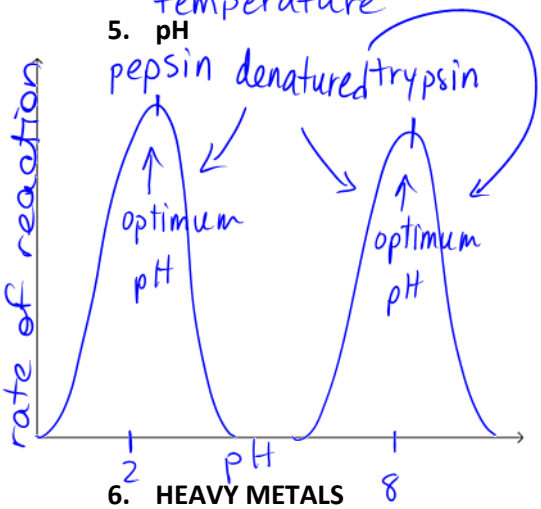
Denaturation = loss of normal shape of an enzyme (protein) and therefore, the ability to form an enzyme-substrate complex.

4. TEMPERATURE



= A rise in temperature results in an increase in enzyme activity. A temperature increases, increased kinetic energy causes more collisions between the enzyme and the substrate molecules and more active sites are filled. As temperature increases further, the reaction rate levels off (optimum temperature) and then declines rapidly because the enzyme is denaturing (high temperatures disrupt the hydrogen bonding between the amino acids).
Recall: a denatured enzyme has lost its normal shape and therefore its ability to form an enzyme-substrate complex.
The temperature sensitivity of enzymes and other proteins contributes to the lethal effects of excessive temperatures.

5. pH



= Another factor that affects enzyme activity is pH. Each enzyme has an optimal pH which helps to maintain the enzyme's tertiary structure (helps to maintain the normal interactions between the R-groups of the amino acids within the enzyme). A change in the pH from optimum can alter the shape of the enzyme and denaturation eventually occurs (either side of the bell curve). Without its normal shape, the enzyme is unable to combine efficiently with its substrate.
For example, pepsin, an enzyme found in the stomach, acts best at an acidic pH of 2, while trypsin, an enzyme found in the small intestine, prefers a basic pH of about 8.

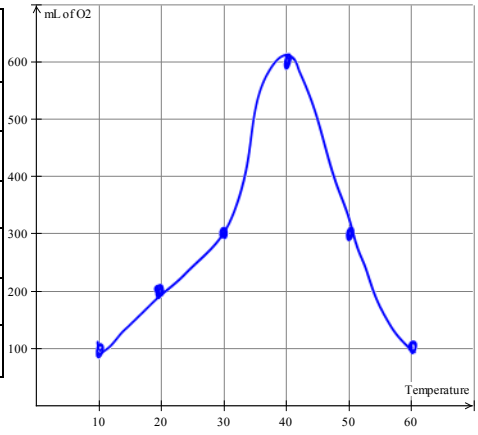
6. HEAVY METALS

- Heavy metals such as lead (Pb^{+2}) or mercury (Hg^{+2}), bond with proteins and thereby inactivate them.

SAMPLE TEST QUESTIONS:

1. Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen.
   a. Plot the results of the data shown to the right in the accompanying graph (only the collected data).

Milliliters of Oxygen formed per minute	Temperature (°C)
100	10
200	20
300	30
600	40
300	50
100	60



   b. On the basis of the above graph, at which temperature did the catalase exhibit the greatest activity?
   40° C

   c. Why did the activity of catalase enzyme decrease as the temperature continued to increase above 40°C?
   denaturation occurs.

   d. Explain why hydrogen peroxide can be broken down by the enzyme catalase, but another substrate, such as maltose cannot be broken down by catalase
   Enzymes are very specific, maltose cannot bind to the active site on catalase

2. Study the table on the right.
   a. Which substrate concentration will initially yield the maximum amount of product formed? 30 mg/mL
   b. Explain why the amount of product formed does not increase as the substrate concentration goes beyond 30 mg/mL
      all active sites on enzymes are filled

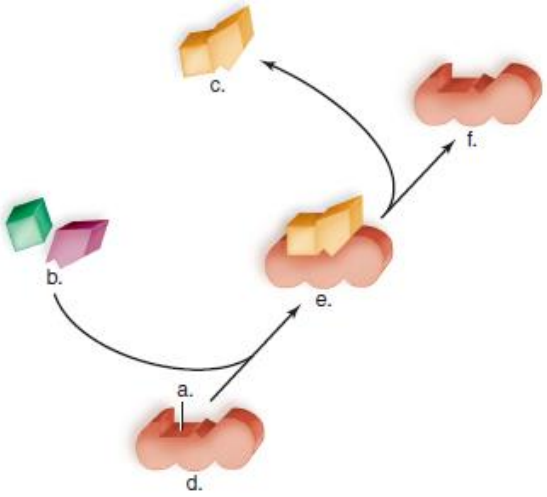
Substrate Concentration (mg/mL)	Amount of Product Formed (mg/mL)
0	0
10	100
20	200
30	400
40	400
50	400

3. Use the following terms to label this diagram:
   - Substrate, enzyme, active site, product, enzyme-substrate complex

a) active site

b) substrate

c) product

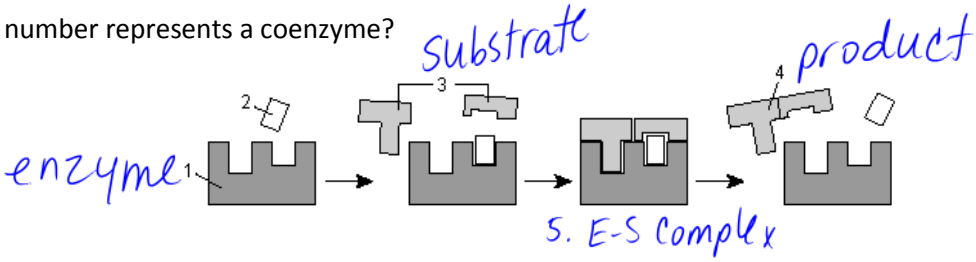


d) enzyme

e) E-S Complex

f) enzyme

4. In the reaction shown, which number represents a coenzyme?
   a. 1
   b. 2
   c. 3
   d. 4



5. Which of the following will cause the denaturation of the protein in egg white?
   a. Adding water
   b. Cooling to 4°C
   c. Heating to 100°C
   d. Removing coenzymes

6. The substrate in the following reaction is:
   Hydrogen peroxide → water + oxygen
   a. Water
   b. Oxygen
   c. Catalase
   d. Hydrogen peroxide

↑ catalase

Biology 12 Name: _____

Enzymes Review

Part One
Provide the best term:

1. The region on an enzyme where it connects to the substrate: active site
2. The non-protein part of an enzyme: cofactor / coenzyme
3. The protein part of an enzyme apoenzyme
4. The energy that is required to get a chemical reaction started: activation energy
5. The sum or total of all the organism's chemical reactions is called: metabolism
6. A molecule that an enzyme bonds to briefly (provide 2 answers): substrate/ cofactor/coenzyme/product
7. An enzyme itself is made up of several larger molecules called: proteins
8. The basic units of these large molecules are amino acids which are linked together by peptide bonds.
9. A molecule that resembles an enzymes substrate and binds to it is called: competitive inhibitor
10. The rate of an enzymatic reaction increases as the temperature increases up to an optimum temperature at which the reaction occurs at the fastest rate. Generally, at around 50°C, the shape/active site of a protein is destroyed. The enzyme is then said to be denatured.
11. Changes in pH may also change the final structure of an enzyme thus affecting the shape (or binding capacity) of the enzyme.
12. This part of the enzyme accepts or contributes atoms to the reaction the enzyme is catalyzing: coenzyme / cofactor / active site
13. This theory states how an enzyme perfectly fits or physically suits its substrate. Induced-Fit/Lock and key
14. After catalyzing a chemical reaction this happens to the enzymes: product released, returns to normal shape
15. Molecules that we call vitamins function as coenzymes in our bodies.
16. How do specific enzymes catalyze their specific chemical reactions?
    The active site for each enzyme is specific to the substrate molecule and will not fit with other molecules

Part Two
Name and describe four factors that affect the activity of enzymes.

- pH
- Temperature
- Amount/Concentration of Substrate
- Amount/ Concentration of Enzyme

Part Three
Make a 15 question study guide based on the notes you have concerning the eight digestive enzymes: salivary amylase, pepsin, bile, pancreatic amylase, trypsin, lipase, peptidase and maltase. Be creative in your questions.

ENZYMES: Self-Test
BC Biology 12 p. 158-165

1. What is metabolism?
the sum of all the chemical reactions that occur in a cell
2. What are the enzymes made of? What is the basic unit of these large molecules? What type of bonds hold these together? Proteins. Amino acids. Peptide bonds

3. Compare and contrast a synthesis and degradation reaction. Degradation: 1 substrate splitting into 2 or more products
Synthesis: 2 or more substrates joining to form 1 product
4. What is Activation Energy? How does an enzyme affect the activation energy?
-the minimum amount of energy required for a reaction to proceed. Enzymes reduce E_a.
5. How do inhibitors, cofactors and coenzymes regulate the activity of an enzyme?
cofactors/coenzymes help substrate bind better to enzyme → ↑ rxn rate. Inhibitors reduce rxn rate.
6. Non-protein, low molecular weight substances, such as magnesium, which are required in certain enzymatic reactions are called cofactors.

7. Non-protein organic molecules may also play crucial roles in enzyme catalyzed reactions. NAD is an example of such a coenzyme.

8. Explain how the following factors can influence the speed of a chemical reaction:

   a. Temperature- as temp. increases, kinetic energy increases causing more collisions to occur → increases rate of chemical rxn.

   b. pH- each enzyme has a preferred pH at which the rate of reaction is the highest

   c. Amount of Substrate- enzyme activity increases as more substrate is added because there are more collisions between substrate + enzyme

9. Pepsin is an enzyme that breaks down protein. You are given a test tube that contains pepsin, egg white (protein), and water. What conditions would you suggest to ensure digestion of the egg white?
pH of 2 (acidic conditions) add HCl
warm temperature

10. Competitive inhibition occurs when a compound inhibits a reaction by (temporarily) occupying the active site of the enzyme and displacing the substrate. This type of inhibition depends on the shape/structure of each kind of molecule present.

Name: _____
Date: _____ Blk: _____

Enzymes Review Worksheet

Part A: Define the following terms in your own word. Be clear and concise!

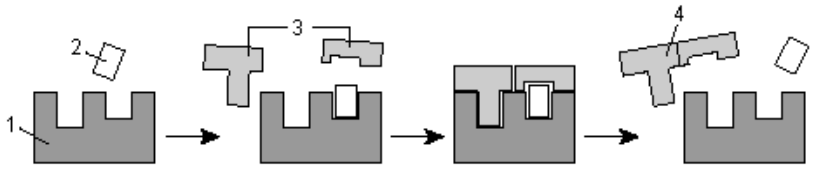
metabolism	
substrate	
enzyme	
active site	
coenzyme	
product	
activation energy	

Part B: Short Answers

1. The equation ADP + $P_i \rightarrow$ ATP is energy (*requiring or releasing*) _____.
2. In the pathway below, the letters stand for _____and the numbers stand for _____. Each and every reaction in a cell requires a specific _____.

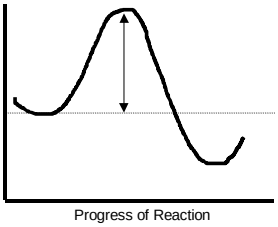
A **1** ⇨ **B** **2** ⇨ **C** **3** ⇨ **D** **4** ⇨ **E**

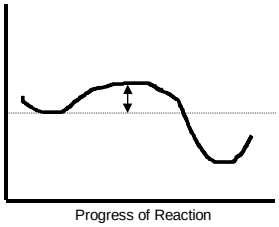
3. If an enzymatic reaction is heated *gently*, it will _____.
4. Enzymes _____the amount of activation energy necessary for a reaction to take place by putting its substrates on a precise "collision course."
5. In the equation S + E $\rightarrow$ SE $\rightarrow$ P + E, what do the letters stand for?
   S: P:
   SE: E:
6. Name two environmental factors that can change the shape of an enzyme.
   i. ii.
7. Name two factors that can speed up enzymatic reactions
   i. ii.
8. Enzymes have helpers called _____. A common example of the latter is NAD.
9. Label the parts on this diagram.



1	
2	
3	
4	

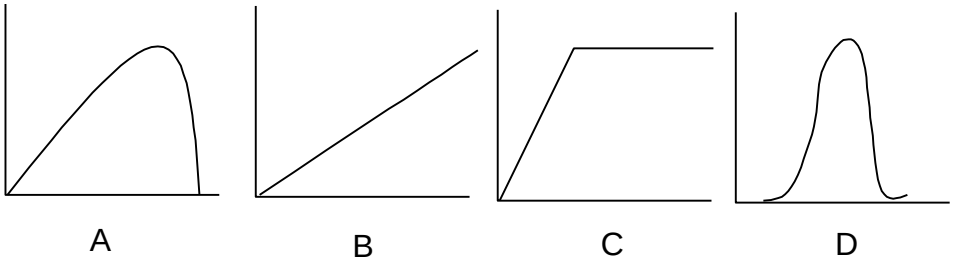
10. Label all missing parts on the graphs below. **Highlight the energy of activation** on both graphs.





11a. Which graph below best represents a graph of the enzyme activity vs pH? Explain what is occurring at each portion of the graph.

11b. Which graph below best represents a graph of the enzyme activity vs temperature? Explain what is occurring at each portion of the graph.



A B C D

Part C: Answer on separate sheets of paper, in your own words.

1. What gland produces the hormone thyroxin? What is the function of thyroxin in metabolism?
2. Explain, using a good example, how a metabolic pathway can be self-regulating (that is, how it can shut itself on and off)
3. How does the "Lock and Key" theory of enzyme action differ from the "Induced Fit" theory? Use diagrams to help your explanation.
4. Why do you think each enzyme has its own preferred pH at which it operates?
5. What is the effect of lowering the temperature on enzyme activity? How about raising the temperature? Draw a graph to show these relationships.
6. Describe three factors that can lead to the denaturing of enzymes. How would denaturing an enzyme affect its activity?
7. What happens to the rate of product formation if you continue to add to an enzyme-catalyzed reaction the following: a) substrate b) enzyme c) an inhibitor d) Lead, mercury, or cadmium e) H^+ ions f) OH^- ions
8. Explain, using a diagram, how competitive inhibitors work and the way they act on enzymes.
9. Discuss, using examples, the effects of reversible and non-reversible inhibitors on enzyme activity.
10. Explain the role of vitamins in metabolic reactions. List at least 2 examples.
11. An experiment was carried out to investigate the action of the enzyme catalase from homogenized (broken down) liver cells. Catalase breaks down hydrogen peroxide (found in liver cells) into oxygen and water ($2H_2O_2 \rightarrow 2H_2O + O_2$). Five test tubes were set up as shown on the table below. The following steps were performed:
    i. equal portions of liver homogenate were placed in the five test tubes.
    ii. the test tubes were placed in the water baths at the temperature indicated in the table for 5 minutes.
    iii. 10mL of hydrogen peroxide was added to teach test tube.
    iv. the amount of oxygen released was recorded

TUBE	**1**	**2**	**3**	**4**	**5**
Temperature (º C)	18	28	38	48	80
mL of O_2 produced in 1 min.	4.1	8.8	18	1.2	0

    a. The more H_2O_2 that is broken down, the _____ (worse/better) the enzyme catalase must be working.
    b. The amount of H_2O_2 that is broken down is directly proportional to the amount of _____ that is produced, so by measuring how much _____ is produced, we can have a reliable measure of how well the enzyme catalase is working.
    c. Explain why there was a difference in the amount of oxygen produced between test tubes 3 and 4.
    d. Account for the low level of oxygen in test tube 1.
    e. Why was there no oxygen produced in test tube 5?
    f. Rank the test tube where the enzymes worked the best to the least.
    g. Explain the results in the 5 test tubes (ie. why was it so slow or fast?)