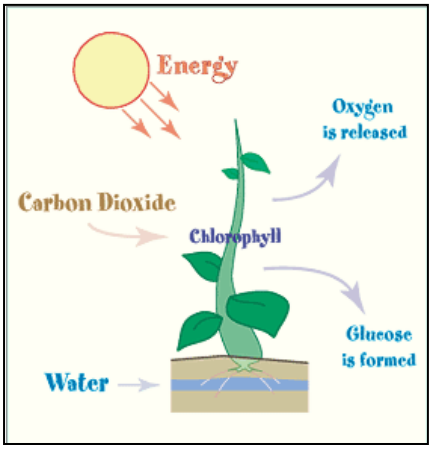
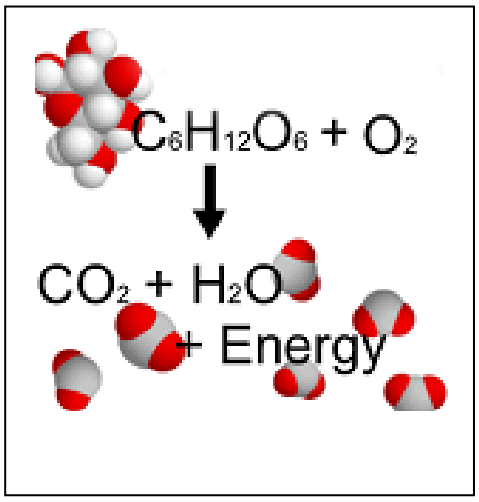
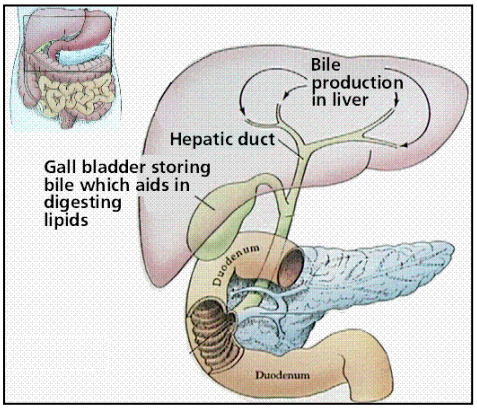
❖ INTRODUCTION

The given pictures represent certain biochemical processes:

Identify the process and list two other processes.





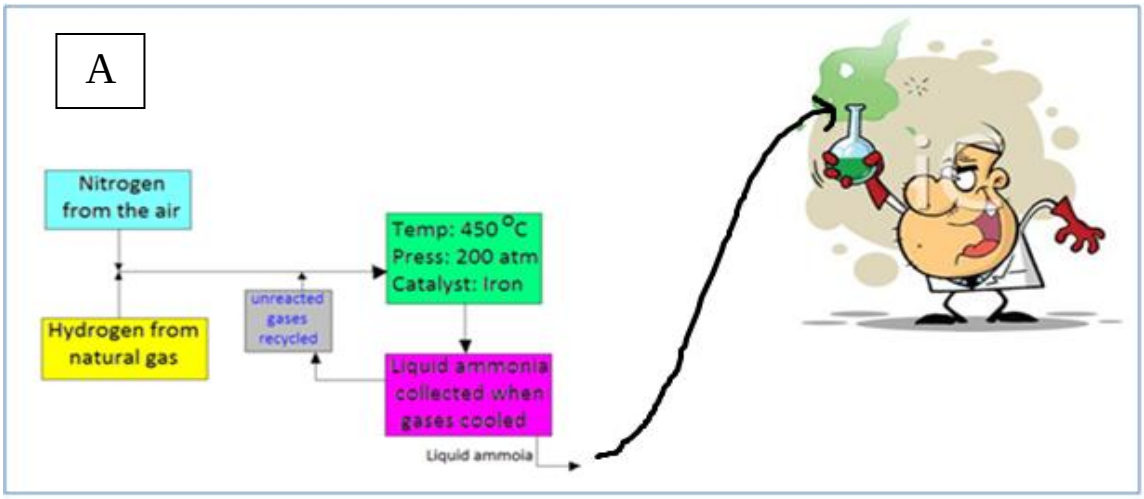


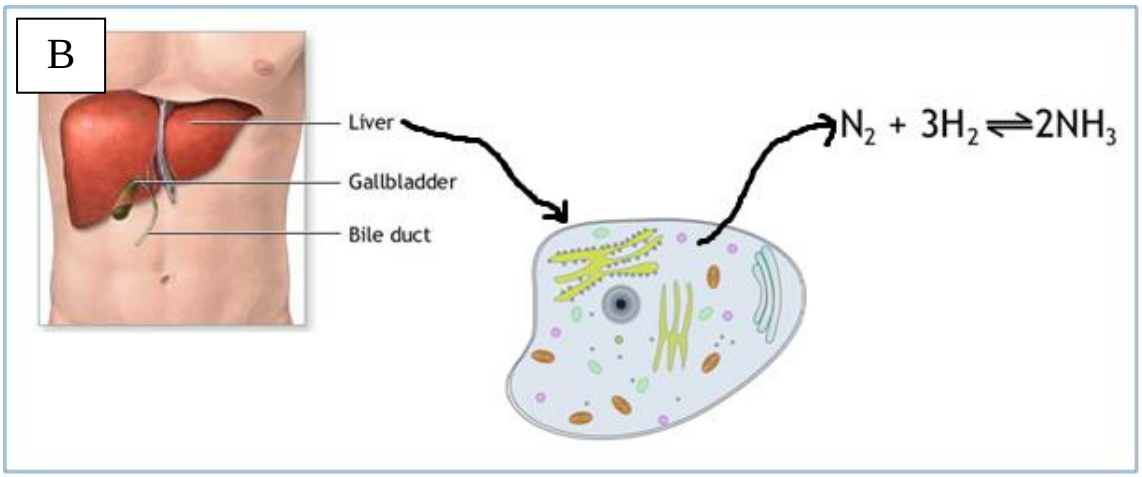
a).....

b).....

❖ COMPARING A BIOCHEMICAL AND PHYSICAL PROCESS

Given below are two chemical reactions showing synthesis of ammonia in two different conditions.





What is the difference in the two reactions?

……

……

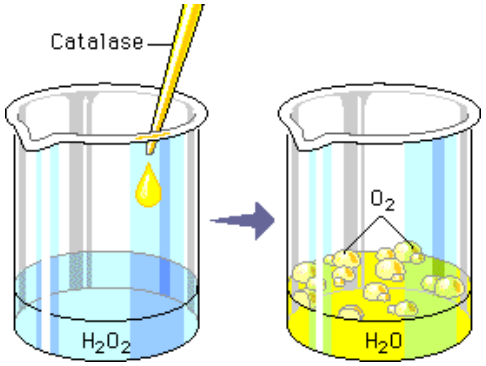
How is ammonia formed in liver cell without the conditions as in reaction A?

……

……

❖ A CHEMICAL PROCESS INVOLVING ORGANIC AND INORGANIC CATALYST.

Another biochemical reaction is the decomposition of H_2O_2. Many organisms can decompose hydrogen peroxide (H_2O_2) enzymatically. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as *catalysts,* substances that speed up chemical reactions without being destroyed or altered during the process. Enzymes are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second.



H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H2O2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably.

1. Experimental evidence.....?

Requirements

Make a list of requirement according to given protocol.

.....
.....
.....

Procedure

1. Take 5 test tubes. Mark them as A, B, C, D and E.
2. Put 2 ml of H_2O_2 (hydrogen peroxide) in each tube and place them in test tube stand.(Use dropper)
3. Place all the test tubes in water bath at 37^0C.
4. Test tube A forms control with only H_2O_2.
5. In test tube B add given amount of MnO_2.
6. In test tube C add given amount of banana tissues.
7. In test tube D add given amount of onion tissue.
8. In test tube E add given amount of potato tissues.
9. Watch for a reaction. Use a scale of 1-5 to rate the reaction. 1 is very little or no reaction and 5 is a large reaction.

10. Record your observations in given table.

❖ **Observations**

	Test tube	Rate of bubbles evolved
1	A (Control)	
2	B (MnO_2)	
3	C (Banana)	
4	D (Onion)	
5	E (Potato)	

❖ Collect data from other groups in given table.

	T1	T2	T3	T4	T5
A (Control)					
B (MnO_2)					
C (Banana)					
D (Onion)					
E (Potato)					

❖ Enter the data in excel sheet and plot a bar graph. Take a print and paste it in given space.

❖ Answer the following questions.

1. Which test tube shows maximum production of oxygen?

   .....

2. Why there is less oxygen production in test-tube A..?

   .....

   .....

3. Is there any difference between reactants of C, D, E and B?

   .....

   .....

4. What is the similarity in the reaction between C, D, E and B.?

   .....

   .....

5. Mention the role of MnO2 in test tube B.

   .....

6. Why do plant and animal tissues (C, D and E) show a similar result as B.?

   .....

7. Can you guess the name of the substance present in potato or liver tissues that cause the decomposition of H_2O_2?

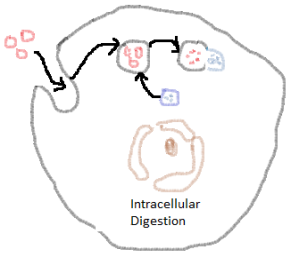
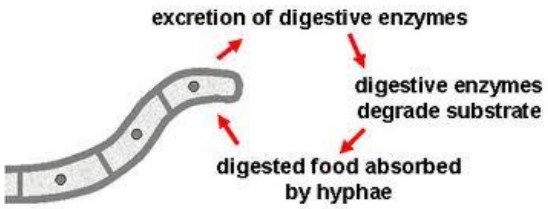
   .....

❖ GOOGLE THE FOLLOWING ABOUT ENZYMES:

1. Discovery and scientist associated.

	Name: Contribution:
Year	
	Name: Contribution:
Year	

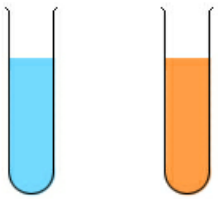
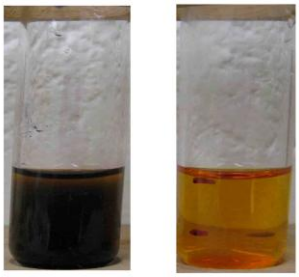
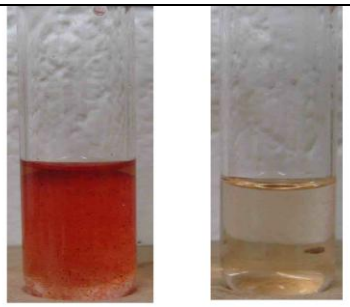
2. Intracellular (Endozymes) and extracellular enzymes (Exozymes)

❖ CHEMICAL NATURE OF ENZYMES

Are enzymes carbohydrates, proteins or lipids.....?

2. Experimental evidence: ……..?

Are enzymes carbohydrates.....? (Benedict and iodine test)		
Test	Observation	Result (Tick your result)
		
		
Are enzymes lipids.....? (Sudan test)		
		
Are enzymes proteins.....? (Biuret test)		
		

Conclusion: Enzymes are.....

❖ HOW ENZYMES WORK.....?

Access the given links.

Animation: 1

http://www.dnatube.com/video/2073/Function-structure-of-enzymes

Animation: 2

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

a) Name the term for part which combines with enzyme? .....

b) Where does the reactant fit in an enzyme? .....

c) What is special about the part where the reactant fits?

.....

.....

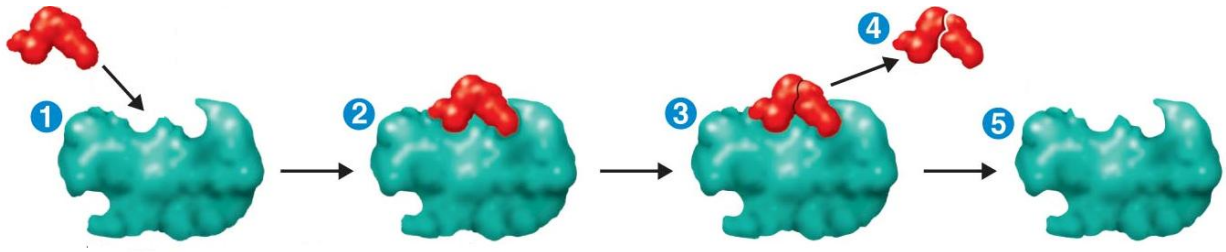
d) What do you mean by substrate? In animation -1 which molecule acts as a substrate?

.....

.....

e) Mark the following terms on given enzymatic process.

Enzyme, substrate, active site, enzyme substrate complex, product



f) By using following symbols write an equation above process.

E- Enzymes

S- Substrate

ES- Enzyme substrate complex

P- Product

❖ ENZYMES AND ACTIVATION ENERGY……..?

3. Experimental evidence…?

Starch is polysaccharide which can be hydrolysed by heating or by an enzyme. Amount of product can be tested by performing Benedict test.

Test	Observation	Inference
Test tube A with 5 ml starch solution. Perform Benedict test		
Test tube B with 5 ml starch solution. Add benedict solution. Place it in water bath for 6 mins.		
Test tube C with 5 ml starch solution. Add benedict solution. Place it in water bath for 12 mins.		
Test tube D with 5 ml starch solution. Add benedict solution. Place it in water bath for 18 mins.		
Test tube E with 5 ml starch solution. Add 5 ml amylase. After five minutes perform Benedict test.		

a) How many test tubes give positive benedict test?

……..

b) Why does test tube '**A**' show negative benedict test?

……..

c) In which test tube do you find more amount of hydrolyzed sugar (as ppt.)?

……..

d) Explain the changes observed in test tube B, C and D. (effect of heating)

……..

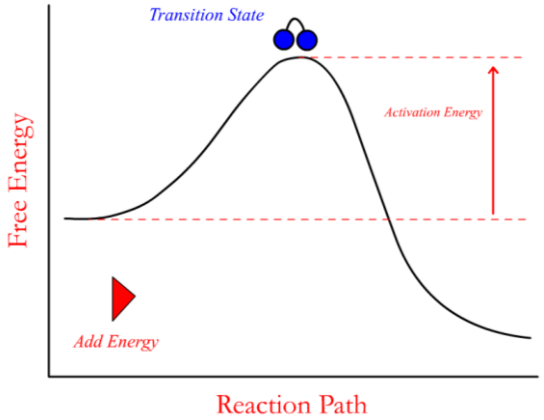
……..

e) Without heating test tube '**E'**, how did it show positive benedict test?

……..

Animation: 3

http://www.stolaf.edu/people/giannini/flashanimat/enzymes/transition%20state.swf



f) Every reaction needs minimum amount of energy to occur. Find the term.

……..

g) What is the difference between free energy of reactants and products?

……..

……

h) 'Enzymes reduce the activation energy'. Redraw above graph to showing change in the curve in presence of enzymes.

❖ COURSE OF AN ENZYME CATALYSED REACTION

In many reactions the substrate will not be converted to a product unless it is temporally given some extra energy, this energy is called activation energy.

One way of increasing the rate of chemical reaction is to increase the energy of reactants by heating them. (As you have performed in experiment -3 in test tubes B, C and D)

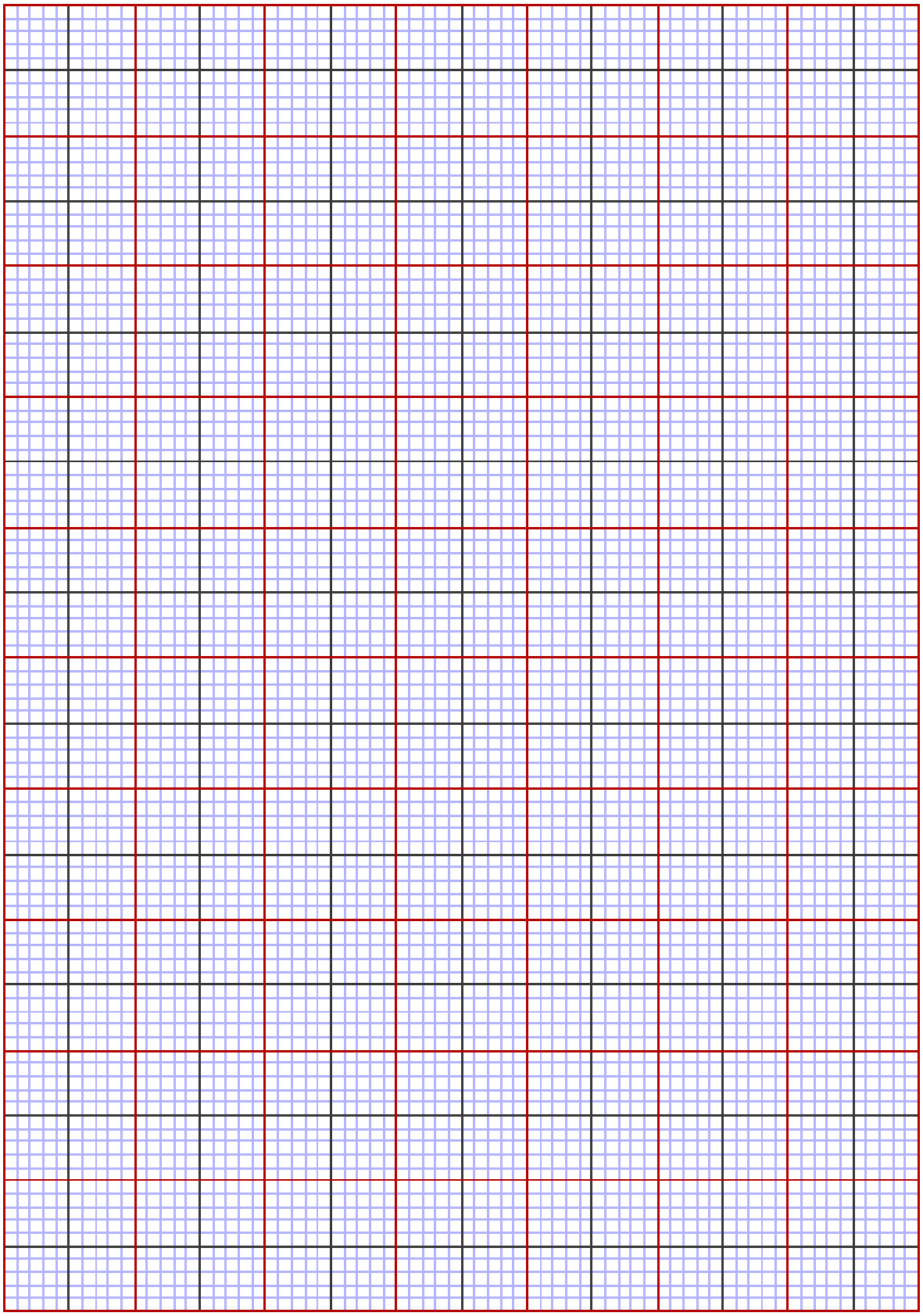
Mammals such as human also use this method of speeding up their metabolic reactions. However the body temperature cannot be raised to above 40^0 C as it causes irreversible damage to many of the molecules.

Enzymes are the solution to this problem because they decrease the activation energy of the reaction which they catalyse. (As you have performed in experiment -3 in test tube 'E')

Given below data shows the result of an investigation using enzyme catalase. (Experiment-1)

Time (Second)	Amount of Oxygen produced (cm^3)
0	2.7
30	4.5
60	5
90	5.4
120	5.7
150	6.3
180	6.6
210	6.8
240	7.0
270	7.2
300	7.4
330	7.5

Use above data and plot a line graph.



a) What do you mean by rate of reaction?

.....

.....

b) On what basis will you measure the rate of reaction in above reaction?

.....

.....

c) Calculate the initial rate of reaction from the graph.

d) Deduce the pattern of rate of reaction from the graph.

.....

.....

e) On what basis will you measure the rate of reaction in following reactions?

i. Starch $\xrightarrow{\text{Amylase}}$ Maltose

ii. $CO_2 + H_2O \xrightarrow[\text{Light}]{\text{Chl}} C_6H_{12}O_6 + H_2O + O_2$

f) Mention the various criterions to measure the rate of different reactions.

❖ THEORIES TO EXPLAIN THE ENZYME SUBSTRATE COMPLEX FORMATION:

Animation: 4

http://www.sumanasinc.com/webcontent/animations/content/enzymes/enzymes.html

Animation: 5

http://www.boardworks.co.uk/media/797600dd/AP%20Biology%20Sample/3_2_induced_fit_animation.swf

❖ GOOGLE THE FOLLOWING :

A. Lock and key hypothesis

Scientist:

Explanation

B. Induced fit hypothesis

Scientist:

Explanation:

❖ CAN ENZYMES ACT ON MORE THAN ONE SUBSTRATE.....?

Experiment -4

Read the given table predict the possible observation), perform the test and verify your prediction.

Test tube	Test	Prediction *(Positive or negative iodine test)*	Observation	Do prediction matches observation
A	2 ml starch, leave it for ten minutes and add 2 ml iodine solution.			
B	2 ml starch + 2ml lipase, leave it for ten minutes and add 2 ml iodine solution.			
C	2 ml starch + 2ml amylase, leave it for ten minutes and add 2 ml iodine solution.			
D	2 ml starch + 2ml protease, leave it for ten minutes and add 2 ml iodine solution.			
E	2 ml starch +2ml sucrose, leave it for ten minutes and add 2 ml iodine solution.			

a) What do you infer by positive and negative iodine test?

.....

.....

b) Though enzymes were added in test tube B, C, D and E. Why did you observe negative result in test tube C only?

.....

.....

c) Why enzymes are not catalysing the process in test tubes B, D and E? (*Hint : Active site*)

.....

.....

.....

.....

d) Mention the conclusion based on above observations.

.....

.....

e) Google the enzymes and products for the following substrates.

Substrate	Enzymes	Products
Sucrose		
Maltose		
Lactose		
Cellulose		
Lipids		
Nucleic acid		
Casein		

One of the properties of enzymes is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

1. Absolute specificity - the enzyme will catalyze only one reaction.
2. Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
3. Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
4. Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

❖ ENZYMES AND pH.....?

Experiment -5

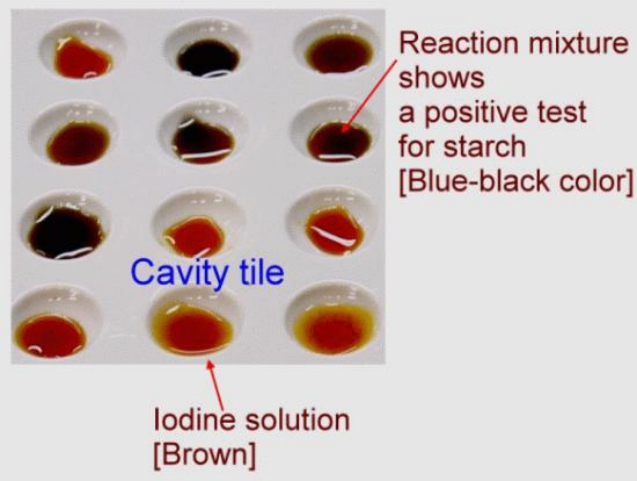
1) Take two beakers and label them as '**A'** and '**B'.**
2) Collect 10 ml saliva in beaker A.
3) Have a sip of lemon juice and immediately collect 10 ml of saliva in beaker B.
4) Place the beakers in the incubator for 5 minutes. (37^{o}C).
5) Take three test-tubes, label them as X, Y and Z.

Test tube			0 min	5 mins	10 mins	15 mins
X	Add 5 ml starch.	Perform iodine test as per instructions given below.				
Y	Add 5 ml starch+ 5 ml enzyme from beaker A.					
Z	Add 5 ml starch + 5 ml enzyme from beaker B.					

6) Immediately with the help of a dropper take a drop from tube X to the tile and then add one drop iodine.
7) Note the time of adding as 0 minute reading. After an interval of 5 minutes,follow the same procedure. Keep on repeating it after an interval of every 5 minutes till 15 minutes.
8) Repeat the same for set Y and Z simultaneously.

Compare the colours with given picture and use following words depending on the range of colour.

Blue black, dark brown, lighter brown, orange brown



1. Write the chemical reaction of above experiment.

2. Write the substrate, enzyme and products of this chemical reaction.

   .....

   .....

3. Why there is positive iodine test in test tube '**Z'** though enzymes was added in it?

   .....

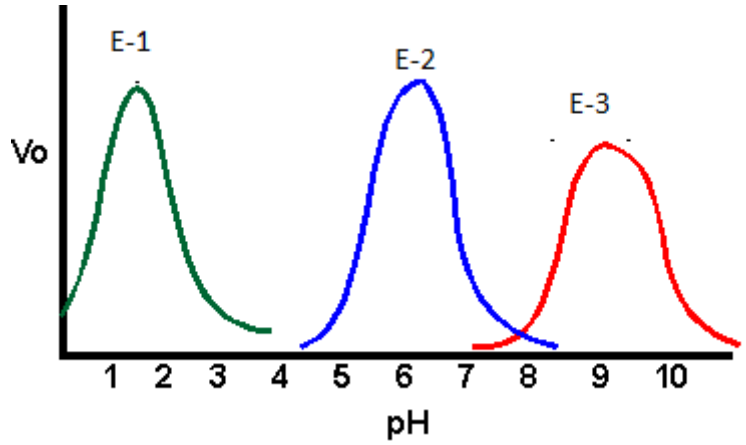
   .....

4. Why was salivary amylase unable to hydrolyse starch in test tube Z? (Hint pH)

   .....

   .....

5. Given graph is showing relation between rate of enzyme activity and pH.



   a) What are the suitable pH range of enzymes E1, E2 and E3?

      .....

      .....

      .....

   b) Give the optimum pH of all three enzymes.

      .....

      .....

      .....

   c) Define optimum pH.

      .....

      .....

      .

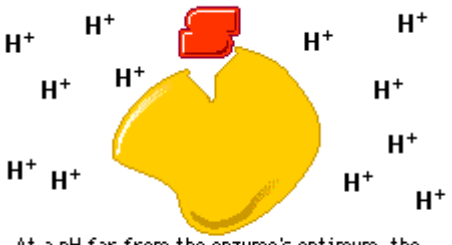
6. Google the optimum pH for the below mention enzymes.
    a) Pepsin.....
    b) Carboxypeptidase.....
    c) Pancreatic amylase.....
    d) Rennin.....
    e) Urease.....
    f) Alkaline phosphatase.....
    g) Catalase.....

Conceptual summary

What happens at different pH can be explained in terms of the shape and structure of the enzyme molecule.

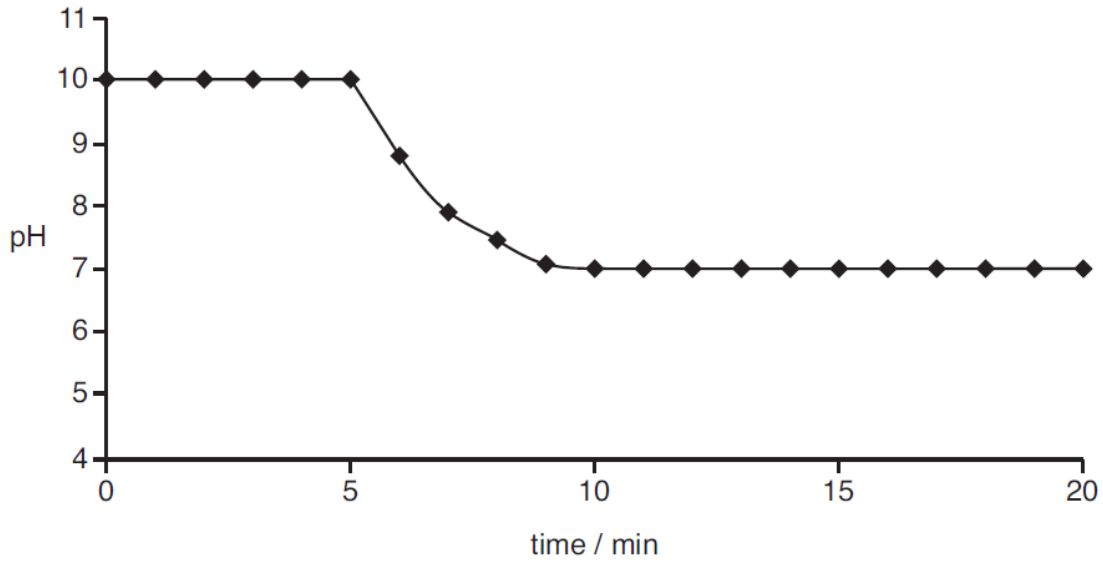
As a type of protein, enzymes are easily affected by changes in pH. At their optimum pH (in the case of amylase, this is a pH of 8), the shape of the enzyme is such that the active site can fit perfectly with the substrate. As the pH decreases from, or increases from the optimum, the acid or base conditions begin to disrupt some of the hydrogen bonds between loops of the protein chains. If the disruption occurs at or near the active site, the active site becomes distorted and substrate can not fit perfectly. Thus not all enzymes in the solution will be able to catalyze their reaction. With increasing or decreasing pH, more enzymes become denatured, and fewer enzymes are able to form that enzyme-substrate complex. The reaction rate continues to decrease. At some point, all the enzymes are denatured, and the reaction rate falls to zero.

Optimal pH



At a pH far from the enzyme's optimum, the shape of the active site changes so severely that the enzyme cannot function.

The enzyme lipase catalyses the hydrolysis of ester bonds in triglycerides. As the reaction proceeds there is a decrease in pH. The progress of the reaction may be followed by using a pH meter.
A solution containing tristearin was placed in a water bath at 25 °C. When the solution had reached this temperature, lipase was added and the mixture stirred. The pH of the reaction mixture was recorded every minute for 20 minutes. The results are shown in figure



a) Using the data in above figure, state the time when

i. lipase was added;

.....

ii. the reaction ended.

.....

iii. Explain why the pH decreases during this reaction.

.....

.....

iv) Find the optimum pH of lipase and represent it with the help of graph.

❖ ENZYMES AND SUBSTRATE CONCENTRATION

Experiment: 5

a) Take four different concentrations of hydrogen peroxide in chambers A, B, C, D and E.

b) Add catalase (5gm grated potato) in chamber A.

c) Immediately connect oxygen sensor with chamber A.

d) Measure the amount of oxygen produce by using data logger at the regular intervals of 30 seconds.

e) Repeat the same for chambers B, C, D and E.

Concentration of hydrogen peroxide	Amount of oxygen produced (per 30 seconds)								
	0 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
A (0%)									
B (0.3%)									
C (0.7%)									
D (1.5%)									
E (3.0%)									

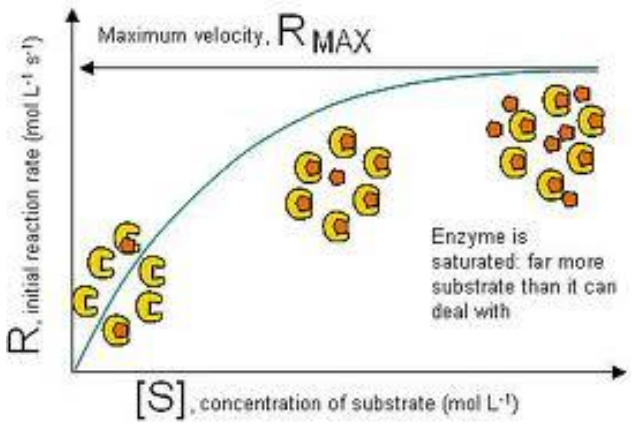
1. Describe the observations.

2. Explain the observations.

Take the print of graph from data logger and paste in given space.

Conceptual summary:

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.



Graph showing relation between different substrate concentrations and enzyme

ENZYMES AND TEMPERATURE.....?

Experiment: 6

- You are provided with 5% solution of an enzyme.
- Take 12 ml of the 5% solution of enzyme and divide it equally in two test tubes X and Y.
- Keep the test tube X in a boiling water bath for 15 minutes. (Control).
- Divide the remaining solution of test tube Y in 3 test tubes Y1, Y2 and Y3.
- Take three 250 ml beakers. Label them as A, B and C. Pour approximately 150 ml of water into each beaker and set temperature as: Beaker A at 0-5^{0}C (ice bath), Beaker B at 35-40^{0}C and Beaker C at 75^0-80^{0}c.
- Place Y1, Y2 and Y3 in beakers A, B and C respectively.
- Set up six test tubes. Label them as A1, A2, B1, B2, C1 and C2.
- In each of the above 6 test tubes add

  2 ml of 1% starch solution.

  1ml of 1% NaCl

  1ml of buffer pH 6.8

- Keep the test tubes labelled

  A1 and A2 in beaker A

  B1 and B2 in beaker B

  C1 and C2 in beaker C

- In the test tubes A1, B1 and C1 add 1 ml of the enzyme solution from Y1, Y2 and Y3 respectively.
- In test tubes A2, B2 and C2 add 1 ml of boiled enzyme solution from test tube X.
- Immediately with the help of a dropper take a drop each from these tubes to the tile and then add one drop of iodine.
- Note the time of adding as 0 minute reading. After an interval of 3 minutes follow the same procedure. (Use stop clock immediately for reading)
- Keep on repeating it after an interval of every 3 minutes till the colour of iodine does not change further.

- Note the time taken for all the experimental tubes, maintained at different temperatures, till they do not give any colour with iodine. This is known as **achromic point**, i.e., no blue colour with the iodine solution.

- Observation table: Colour change of iodine at different temperature

Time (Minutes)	$0\text{-}5^0C$	$35\text{-}40^oC$	$75\text{-}80^oC$
0			
3			
6			
9			
12			
15			
18			
21			
24			

1. State how long it took for the starch to be completely broken down in tube B1?

   .....

2. From the above table, find at what temperature does amylase enzyme work best?

   .....

3. Why is there positive iodine test in test tubes A2, B2 and C2 till the last reading?

   .....

4. What happen if we use hydrochloric acid instead of sodium chloride solution?

   .....

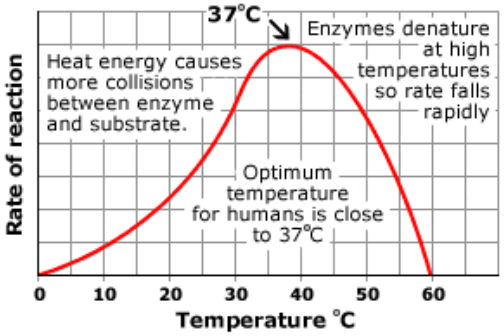
5. What is the relation between temperature and enzyme activity?

   .....

   .....

CONCEPTUAL SUMMARY:

Each enzyme has an optimum temperature at which it works best. A higher temperature generally results in an increase in enzyme activity. As the temperature increases, molecular motion increases resulting in more molecular collisions. If, however, the temperature rises above a certain point, the heat will denature the enzyme, causing it to lose its three-dimensional functional shape by denaturing its hydrogen bonds. Cold temperature, on the other hand, slows down enzyme activity by decreasing molecular motion.

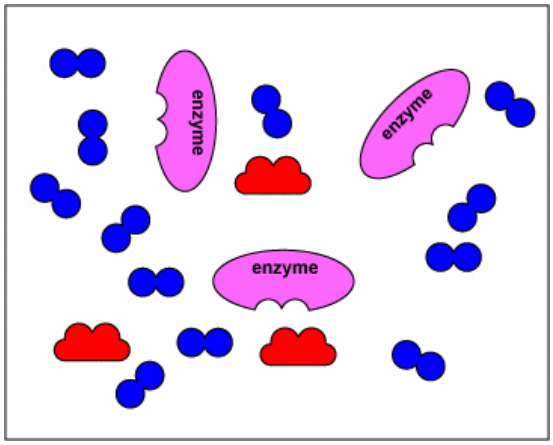


ENZYMES AND INHIBITORS.....?

Experimental evidence : 7

Introduction

Tetrahydrofolate is a co-factor which is needed for the de novo synthesis of thymidine monophosphate, which is required for the biosynthesis of bacterial DNA and RNA. Sulfonamides (such as sulfamethoxazole) and diaminopyrimidines (such as trimethoprim) inhibit different enzymes in the biosynthesis of tetrahydrofolate in the bacteria. Due to the inhibited production of tetrahydrofolate, the bacteria is unable to synthesize the thymidine, and is therefore also unable to produce new DNA or RNA. This eventually leads to the death of the bacteria. Both sulfonamides and diaminopyrimidines act by mimicking the substrate of their respective enzymes, and inhibit the enzyme by blocking the active site of the enzyme.



http://pharmaxchange.info/press/wp-content/uploads/2011/05/competitive-antagonism-of-tetrahydrofolate-biosynthesis-by-sulfonamides-and-diaminopyridines.gif

Animation: 6 http://bcs.whfreeman.com/thelifewire/content/chp06/0602001.html

Animation: 7 http://www.mcgrawhill.ca/school/applets/abbio/quiz/ch06/a_biochemical_pathway.swf

1. What do you mean by enzyme inhibitors?

   .....

   .....

   .....

2. Mention the ways of enzymes activity inhibition?

   a).....

   b).....

   c).....

   d).....

3. Answer the following:

a) Inhibition by denaturation

.....

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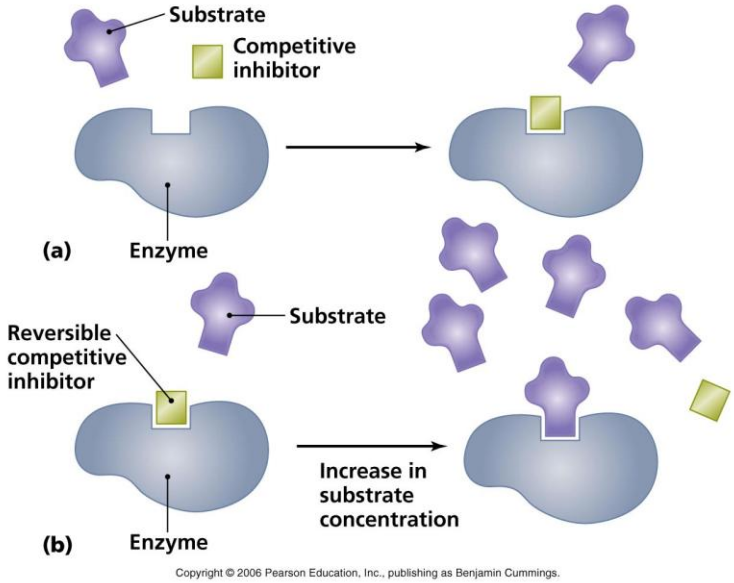
.....

b) Competitive inhibition



i. Define competitive inhibition:

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.....

ii. Why is it so called competitive inhibition?

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.....

.....

iii. Give a method to reduce or reverse the process of competitive inhibition.

.....

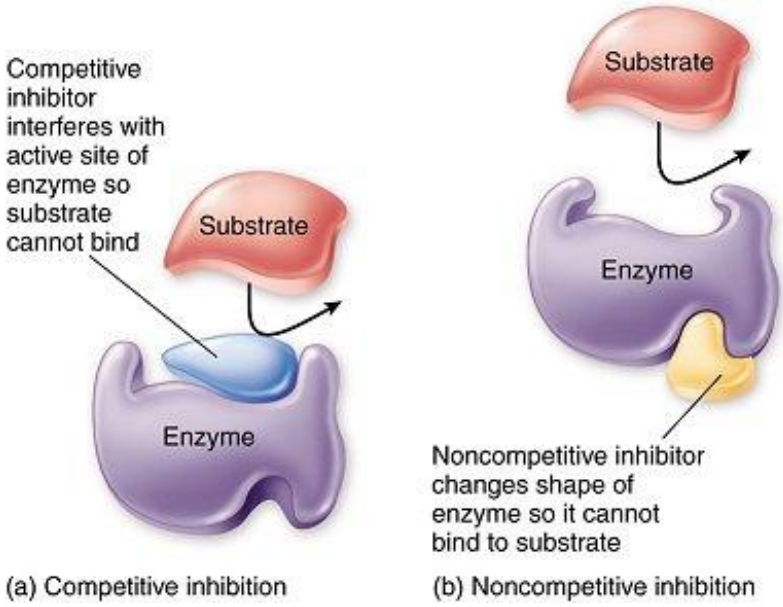
iv. Give any one example of competitive inhibition. (Ref: Introduction)

Enzyme.....

Substrate.....

Inhibitor.....

c) Non- competitive inhibition



i. Define non-competitive inhibition:

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.....

.....

....

ii. Why is it so called non-competitive inhibition?

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.....

iii. Will increase in substrate concentration reduce or reverse the process of inhibition? Why?

.....

iv. Differentiate between competitive and non-competitive inhibition.(Ref: Figure)

.....

.....

.....

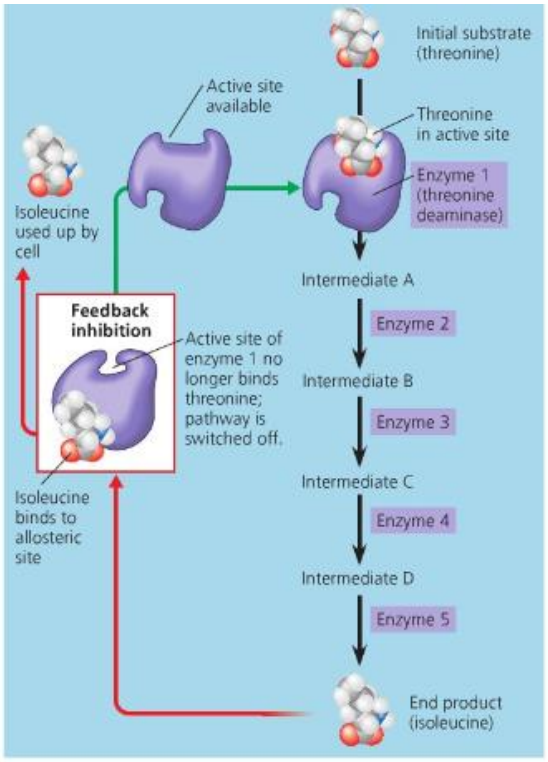
v. Google any one example of non-competitive inhibition.

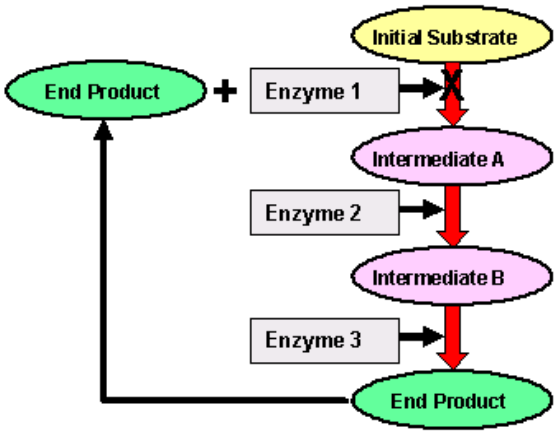
Enzyme.....

Substrate.....

Inhibitor.....

d) End products / feed back or Allosteric inhibition (Animation-7)





i. Define allosteric inhibition:

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.....

ii. Why is it so called feedback inhibition?

.....

.....

iii. What do you mean by allosteric site in enzymes molecule?

.....

.....

iv. Google any one example of allosteric inhibition.

Enzyme.....

Substrate.....

Inhibitor.....

ENZYMES CLASSIFICATION

a) Classify the enzymes on the basis of reaction types.

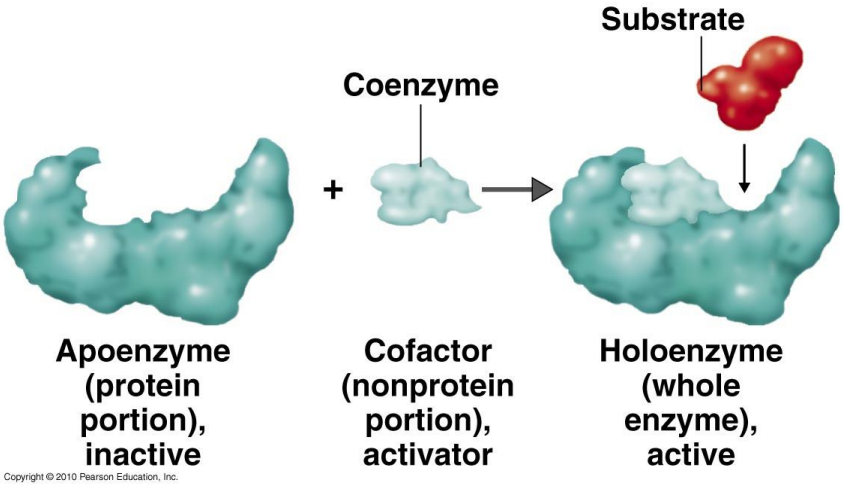
http://academic.pgcc.edu/~kroberts/Lecture/Chapter%205/05-T01_EnzymeClass_T.jpg

Reaction type	Class	Example

b) Substrate acted upon by the enzyme.

Substrate	Enzymes
Protein	
Lipids	
Sucrose	
Nucleic acid	
Urea	
Maltose	
Lactose	

CLASSIFICATION OF ENZYMES ON THE BASIS OF CHEMICAL COMPOSITION:



1. What do you mean by simple enzymes?

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2. What do you mean by conjugated enzymes?

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3. Define the following terms.

a) Apoenzymes

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b) Cofactor

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.....

.....

c) Holoenzyme

.....

.....

4. Write any three examples of coenzymes.

a) .....

b) .....

c) .....

Define the following and give suitable examples:

a) Zymogens or proenzymes

.....

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b) Isoenzymes or isozymes

.....

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.....

c) Ribozymes

.....

.....

INDUSTRIAL APPLICATIONS OF ENZYMES

1. .....
2. .....
3. .....
4. .....
5. .....

CONCLUDE THE PROPERTIES OF ENZYMES.

Experiment	Properties
Experiment:1	
Experiment:2	
Experiment:3	
Experiment:4	
Experiment:5	
Experiment:6	
Experiment:7	

WEB LINKS

http://emp.byui.edu/wellerg/Molecules%20of%20the%20Cell%20Lab/instruction/Molecules%20of%20the%20Cell%20Instructions.html (Food tets)

http://www.mcgrawhill.ca/school/learningcentres/mod/resource/view.php?id=21062 (virtual lab exercise)

http://www.worthington-biochem.com/introbiochem/discoveries.html (details)

http://www.ucl.ac.uk/~ucbcdab/enzass/substrate.htm (Km value)

http://academic.pgcc.edu/~kroberts/Lecture/Chapter%205/05-T01_EnzymeClass_T.jpg (enzyme class)

http://apchute.com/ap1int.htm (cell membrane)

http://academic.pgcc.edu/~kroberts/ (softwares)