Laboratory 2: Enzyme Catalysis

*graph data from an enzyme experiment
*determine the rates for enzymatically catalyzed reactions
*discuss a method for determining enzyme activity
*discuss a relationship between dependent and independent variables
*discuss the effect on initial reaction rates produced by changes in temperature, pH, enzyme concentrations, and substrate concentrations
*design an experiment to measure the effect on enzyme activity produced by changes in temperature, pH, enzyme concentration, and substrate conc.

enzymes = large, tertiary or quartenary proteins with active sites where substrate(s) fit to allow accelerated catalysis of chemical reaction
Catalase, present in most cells, breaks down poisonous hydrogen peroxide H_2O_2 to H_2O and O_2 (gas bubbles). To determine the "rate of reaction", you measure the disappearance of reactants or the production of products over a period of time.

Procedure
Throughout the lab, measured amounts of hydrogen peroxide solution are added to a beaker (cup); measured enzyme is added and the reaction allows to proceed X time. To "stop" the reaction, sulfuric acid (H_2SO_4) is added. The solution is then titrated by adding drops or Burette-measured amounts of potassium permanganate....as long as there is peroxide left, it will decolorize the dark purple permanganate to clear! However, when the titration is complete, any excess permanganate will remain dark, and calculations can be made on amount of perman used and/or amount H_2O_2 still present. Since time is known, rate can also be calculated.

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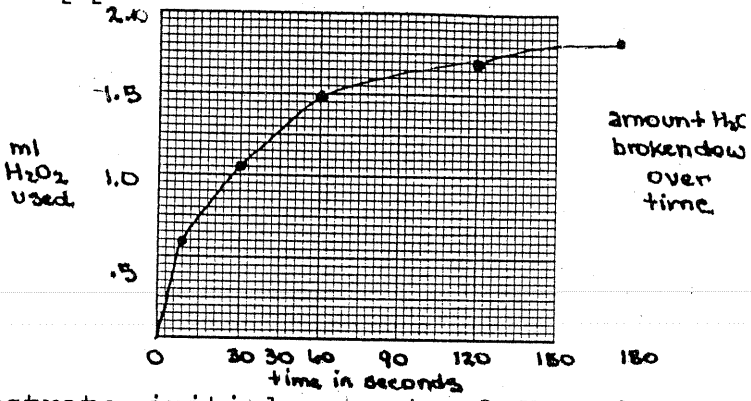
This table and graph shows the results for titration in ml permanganate of the catalase-catalyzed breakdown of H_2O_2 over time intervals 0-180 seconds.

$KMnO_4$ (mL)	Time (seconds) 10	30	60	120	180
A. Baseline = 0 seconds	3.1				
B. Final reading	8.6	10.6	12.2	13.6	14.9
C. Initial reading	6.1	8.6	10.6	12.2	13.6
D. Amount of $KMnO_4$ used (B minus C)	2.5	2.0	1.6	1.4	1.3
E. Amount of H_2O_2 used (A minus D)	0.6	1.1	1.5	1.7	1.8



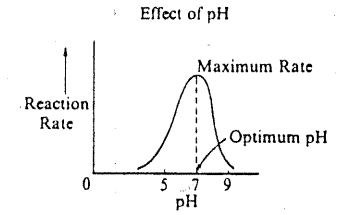
To figure the rates of reaction, substrate initial amount of H_2O_2 from time X amount of H_2O_2, and divide by time X.

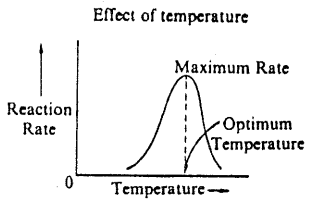
initial rate = (0 ml H_2O_2 used at time 0 − 0.6 ml H_2O_2 used at time 10 sec) / 10 sec = 0.06 ml H_2O_2 per minute

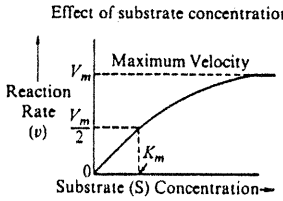
Time intervals (seconds)				
Initial (0-10)	10-30	30-60	60-120	120-180
0 − 0.6 / 10	0.6-1.1 / 20	1.1-1.5 / 30	1.5-1.7 / 60	1.7-1.8 / 60

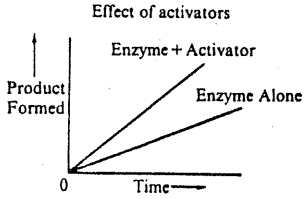
*Reaction rate (mL H_2O_2/sec)

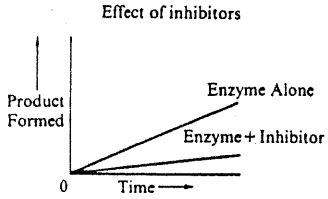
When you get the rates for each interval, you will find that the rate is highest at the start where there are many molecules to be worked on, but drops as substrate decreases (becomes "harder" to find molecules to work on). The reason you use sulfuric acid to "stop" the reaction is that extreme pH changes often denature enzymes. Lowering of temperature will slow enzymes as it does all molecular events, and raising of temperature will speed up the reaction to a point of denaturing and abrupt decline. Many other factors may affect enzymes, as seen below.


Effect of pH
Reaction Rate
Maximum Rate
Optimum pH
0
5
7
9
pH


Effect of temperature
Reaction Rate
Maximum Rate
Optimum Temperature
0
Temperature


Effect of substrate concentration
Reaction Rate (v)
Maximum Velocity
V_m
$\frac{V_m}{2}$
K_m
0
Substrate (S) Concentration


Effect of activators
Product Formed
Enzyme + Activator
Enzyme Alone
0
Time


Effect of inhibitors
Product Formed
Enzyme Alone
Enzyme + Inhibitor
0
Time

Lab 2

BIOLOGY

SECTION II

Time—1 hour and 30 minutes

Answer all questions. Number your answer as the question is numbered below.

Answers must be in essay form. Outline form is NOT acceptable. Labeled diagrams may be used to supplement discussion, but in no case will a diagram alone suffice. It is important that you read each question completely before you begin to write.

1. Genetic variation is the raw material for evolution.
   a. Explain **three** cellular and/or molecular mechanisms that introduce variation into the gene pool of a plant or animal population.
   b. Explain the evolutionary mechanisms that can change the composition of the gene pool.

2. Discuss how cellular structures, including the plasma membrane, specialized endoplasmic reticulum, cytoskeletal elements, and mitochondria, function together in the contraction of skeletal muscle cells.

3. Enzymes are biological catalysts.
   a. Relate the chemical structure of an enzyme to its specificity and catalytic activity.
   b. Design a quantitative experiment to investigate the influence of pH **or** temperature on the activity of an enzyme.
   c. Describe what information concerning the structure of an enzyme could be inferred from your experiment.

Select **two** of the following three pairs and discuss the evolutionary relationships between the two members of each pair you have chosen. In your discussion include structural adaptations and their functional significance.

PAIR A: green algae
vascular plants

PAIR B: prokaryotes
eukaryotes

PAIR C: amphibians
reptiles

END OF EXAMINATION

Free-Response Question 3

Enzymes are biological catalysts.

a. *Relate the chemical structure of an enzyme to its specificity and catalytic activity.*

b. *Design a quantitative experiment to investigate the influence of pH **or** temperature on the activity of an enzyme.*

c. *Describe what information concerning the structure of an enzyme could be inferred from your experiment.*

Scoring Standards for Question 3

Structure and catalytic activity of enzyme (maximum of 4 points)

1 Protein or amino acids (and/or others, such as ribozyme)
1 3-D shape/levels of structure (primary, secondary, tertiary, etc.)
1 Bonding explanation of structure (alpha helix, hydrophobic interactions, van der Waals forces, etc.)
1 Active site ("groove", "pocket")/special shape for substrate/"lock and key"
1 Modifers of enzyme shape (cofactors, activators, inhibitors)
1 Induced fit theory (function of enzyme - substrate fit)
1 Activation energy lowered
1 Substrate altered

Experimental design (maximum of 5 points)

Experiment based on enzymatic activity (initial choice of temperature or pH is binding)

1 Eliminate other variables (conc., amounts, time, pH, temp. in alternate experiment)
1 Negative control (setup without enzyme or without substrate)
1 Describe experimental variable (temperature or pH) values or range
1 Uses correct enzyme-substrate pair
Measure disappearance of substrate, appearance of product, heat production, etc.
Report data (predicted results, such as loss of activity, reduced activity, or no change in activity)
1 Elaborate on experiment (exemplary set-up; independent, dependent variables identified; rate calculation or explanation; replication of experiment, etc.)

Inference from experimental design (maximum of 2 points)

1 Correct link of predicted results to changes in enzyme structure
a. range of activity implies slight change in shape OR
b. loss of activity implies denaturation OR
c. no loss in activity implies no change in structure
1 Elaboration on changes in enzyme structure (conformation explanation, bonding shifts, or an explanation of why no change in activity is predicted)

a. Enzymes are proteins and ~~[illegible]~~ the structure of all proteins is dictated by the sequence of amino acids used to form the protein. This is known as primary structure. Hydrogen bonding of the amine and carbonyl groups of the amino acids produces secondary structure in the form of alpha-helices or beta-pleated sheets. Hydrophobic interaction of the R groups of the amino acids, disulfide bonds of cysteine components, and the unusual kinks in shape caused by the amino acid proline causes tertiary structure. Quaternary structure is dictated by the interaction with other polypeptide chains of the protein.

Thus the three-dimensional structure of the protein is formed. ~~[illegible]~~ This includes an active site, often aided by cofactors (inorganic ions) to attract substrate (reactant) molecules to the enzyme. Enzymes are specific to only one or a few substrates and the shape of the substrate and active site yields an induced fit due to the weak bonding of the two. Sometimes enzymes also have ~~[illegible]~~ a regulatory site as well, in which case it would be an allosteric enzyme. The regulatory site binds to either inhibitors or stimulators which either change the configuration of the active site to either discourage or promote the binding of substrate.

Sometimes an enzyme will, due to the shape of
s active site, bind to more than one type of substrate.
or example, in photosynthesis, ~~the carboxylase~~ RuBP
arboxylase ~~binds~~ will cause ribulose bisphosphate
nd CO_2 to yield two 3-carbon compounds (PGAL-
hosphoglyceraldehydes). But the carboxylase sometimes
inds to O_2 (oxygen) instead of CO_2, in which
ase glycolic acid is formed. This is called
hotorespiration.

. An experiment to test the influence of pH ~~of~~ on
n enzyme ~~of the same enzyme~~ would first need an
nzyme and a substrate. An indicator (such as
olor change) would be useful to indicate either
he formation of product or the disappearance of
ubstrate. The reactions would be timed ~~accurately~~
~~by color change or other indicator~~ with a ~~stopwatch~~ chronometer
so that the time between the introduction of substrate
o the enzyme solution and the color change or other
idication of the completion of the reaction would be
n indicator of enzymatic activity. The less time for
e reaction to occur, the ~~greater~~ greater the enzyme's level
ctivity.

I would set up several test tubes containing
dentical concentrations and quantities of substrate, as
ell as several test tubes containing equal concentrations
nd amounts of enzyme, except that each test
ube would also include an identical volume of
solutions of varying pH. The ~~control~~ variable thus,
s the pH of these solutions. The control tube would

containing only the enzyme solution and an equal volume of distilled water.

Test tubes of enzyme and substrate would be mixed, and the reactions would be timed. Then, the time of the reactions would be plotted on a graph versus the pH of the solutions (x-axis). The pH of the solution that yielded the fastest reaction would indicate the best pH level for enzyme activity.

c. A great deal about the structure of the enzyme could be inferred from the results. If the enzyme was most active in acidic solutions (low pH), the H^+ ions in solution may have bound to some anions on the amino acids of the enzyme, causing the shape of the active site to be optimal. If enzyme activity was lowest in acidic solutions, these interactions could have distorted the active sites shape to cause inhibition of the reaction.

Comment: This excellent response received the maximum score of 10. The student mentions 7 eligible points in Part (a) but could only receive the maximum of 4 points. The student states that an enzyme is a protein (1 point), has secondary and tertiary structures (1 point), held together with different kinds of bonds or specific amino acids such as proline (1 point). The student then mentions the active site formation (1 point) which can be altered by cofactors (1 point). The student also mentions the induced fit of the enzyme and substrate (1 point) and provides a description of what an enzyme (RuBPcarboxylase) does to a specific substrate (RuBP and carbon dioxide) and says that it makes a different substance (PGAL) (1 point).

The student received an experimental design point for control of variables, as identical concentrations and quantities of substrate are used. The student also uses a negative control for the experiment, which is set up with only the enzyme. The student uses a range of pH (1 point). There was a way to measure the reaction (1 point), and the experiment merited an elaboration point for noting the independent/dependent variables and also for a clear statement of measurement equipment. The student gives predicted results (1 point), and is able to relate the results of the experiment with loss of activity to a change in the structure of the enzyme with a general statement of distorted active site (1 point) and a clear explanation of bonding changes (1 point).

2000 AP® BIOLOGY FREE-RESPONSE QUESTIONS

BIOLOGY

SECTION II

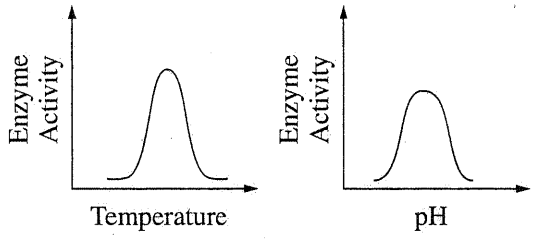
Time—90 minutes

4 Questions

Directions: Answer all questions. Write your answers on the pages following the questions in the pink booklet.

Answers must be in essay form. Outline form is NOT acceptable. Labeled diagrams may be used to supplement discussion, but in no case will a diagram alone suffice. It is important that you read each question completely before you begin to write.

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



   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 the data shown for either temperature or pH. Be sure to state the hypothesis that was tested here.

2. Feedback mechanisms are used by organisms to maintain the steady-state physiological condition known as homeostasis. Choose **three** of the following and for each, explain how feedback mechanisms maintain homeostasis.

   a) Blood glucose concentration.

   b) Calcium ion concentration in blood.

   c) Body temperatures in mammals.

   d) Osmolarity of the blood.

   e) Pulse rate in mammals.

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AP® Biology 2000 — Scoring Standards

Question 1 Scoring Guide

Each bullet is worth one point:

Part a. (maximum 6 points)

- **Optimum** temperature and pH *concept* [must include both temp and pH]
- **Enzyme/Substrate Fit** *concept*
  (function dependent on conformation complementarity between enzyme and substrate)
- **Tertiary** (and sometimes quarternary) structure **determines** function
- Description of enzyme **structure or function**, e.g.

Structure	Function
Elegant description of primary to tertiary or primary to quarternary levels of structure	Increases rate of reaction
Protein folding/coiling	Increases proximity of reactants
Co-enzymes/co-factors	Decreases activation energy of the catalyzed reaction
Zymogens	Decreases time to reach equilibrium
Allosteric effectors	Induced fit and/or orbital steering ("bond stress")

- **Denaturation** *concept* [temp and/or pH] linked to decreased enzyme activity
  (e.g. "denaturation" in context or unfolding or change in 3D shape, **not** "enzyme breaks down")
- **How temperature affects** conformation
  (increased temperature breaks specific bonds, e.g. hydrogen, Van der Waals, disulfide bridges)
- **How pH affects** conformation
  (change in H^+ concentration causes a change in specific bond interactions, e.g. hydrogen; ionic; R-group interactions)
- **Kinetics** (increased or decreased molecular movement) linked to effect on enzyme activity due to increase or decrease in temperature up to the optimum

Part b. maximum 6 points

Experimental design must be relevant to the data shown in the graphs

- **What is measured** (e.g. product formed or substrate used)
- **How is it measured** (titration or spectrophotometry or color change or bubbles counted, etc.)
- The **independent variable** (temperature/pH) is **manipulated** to produce the results [at least 3 data points are identified]
- The described experiment **could produce these data**
  (Experimental design included sufficient range, varied the temp/pH of the reaction mix not the enzyme, what was measured, and how it was measured)
- Held **experimental factors constant** (specified at least one)
- Specified a **control group for comparison** (no enzyme or boiled enzyme or no substrate)
- **Verified** results (e.g. repeated trials; results represent an average)
- **Hypothesis** clearly related to experiment of choice, and clearly identified as a hypothesis; can use the if/then…form.

Lab 2
Enzymes

-4-

1 D1

BIOLOGY

SECTION II

Time—90 minutes

4 Questions

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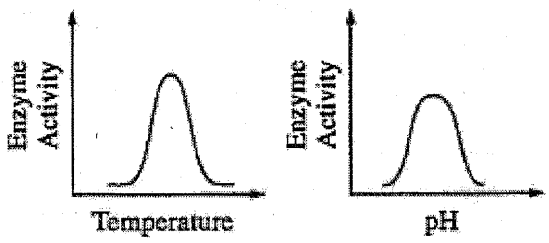

a) How do (1) temperature and (2) pH affect the activity of this enzyme? In your answer, include a discussion of the relationship between the structure and the function of this enzyme, as well as a discussion of how structure and function of enzymes are affected by temperature and pH.

b) Describe a controlled experiment that could have produced the data shown for either temperature or pH. Be sure to state the hypothesis that was tested here.

a. As the results from the experiment show, the enzyme has both an optimal temperature and an optimal pH. Deviation from this optimum results in a decrease in enzyme activity and efficiency. Enzymes are proteins whose unique structure allow them to catalyze chemical reactions. ~~They have primary struct~~ The sequence of amino acids, presense or absense of alpha helixes and beta

GO ON TO THE NEXT PAGE

ADDITIONAL PAGE FOR ANSWERING QUESTION 1

pleated sheets, the types of bonds (ionic, covalent, disulfide, etc.) and the aggregation with another ~~enzyme~~ protein all effect an enzyme's ability to perform. This unique structure results in an active site, an area that is conducive to the reactants of a particular reaction. Each enzyme is very specific and can catalyze only one reaction. Its active site is only open to one substrate (reactant). This is known as the lock and key theory of enzyme specifity. By temporarily hydrogen bonding to the substrate, the enzyme puts the reactants in positions which enhance the probability of a reaction occuring. When heat is applied to an enzyme, the movement of substrate increases and the substrates are more likely to come in contact with the enzyme's active site. This increases enzyme activity. However if too much heat is applied, the enzyme will denature, its shape will change, and the substrate will no longer be able to fit in the active site, decreasing enzyme activity. The same is true for pH. If the pH is too acidic, the influx of H^+ may affect the bonds of the enzyme, as may an influx of OH^- if the pH is too basic. This will

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1 D3

ADDITIONAL PAGE FOR ANSWERING QUESTION 1

also affect the shape of the enzyme's active site and adversely influence its ability to catalyse a reaction.

D. A controlled experiment could have been produced to show the effects of temperature on enzyme activity. The hypothesis would be that enzymes work best at a warmer temperature, but if the temperature is too hot, the enzyme will denature and its activity rate will decrease. In order to do this an enzyme and substrate must be Choosen. A good enzyme would be catalase, which increases the rate at which hydrogen peroxide (H_2O_2) breaks down into water (H_2O) and oxygen (O_2). For the control, the enzyme and substrate would be placed in room temperature. Numerous beakers of enzyme and substrate would be allowed to sit for different time intervals. The reactions could be stopped by the addition of a strong acid to the beakers. This would denature the enzymes and stop the conversion of H_2O_2 into H_2O and O_2. ~~A graph could then be plotted of energ~~ In order to determine how active the enzyme was, a titration could be used. A titration is the introduction of an indicator chemical

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1 D4

ADDITIONAL PAGE FOR ANSWERING QUESTION 1

mat allows one to visualize the endpoint of a reaction. ~~A~~ The amount of H_2O_2 remaining could be determined and plotted on a graph. To check the affects of temperature on enzyme activity, the experiment could be run several times more, but each time with a different temperature, some colder and some warmer, By comparing these various graphs to the graph of the control, it would be possible to determine the affects of heat on enzyme activity

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