

1000 How Poisons Work

This kit focuses on the mechanism of enzyme activity, and how poisons affect that activity. The materials provided in this kit allow up to 15 pairs of students to observe the following reactions:

- the enzymatically catalyzed breakdown of hydrogen peroxide into oxygen gas and water
- the breakdown of starch into glucose as catalyzed by amylase
- the effects of metal poisons on the above reactions
- a comparison between organically and inorganically catalyzed reactions, and their sensitivities to poisons.

The material is suited for high school or advanced junior high school classes of chemistry and biology. The experiments can be performed in one to two 45 minute class periods.

Contents:

LabForms (15)
Razor Blades (15)
Pipets (17)
Chemstrips (1 package)
Dilute solutions of:
 Hydrogen Peroxide (2 - 120 ml bottles)
 Lead Nitrate (2 - 30 ml bottles)
 Mercuric Nitrate (2 - 30 ml bottles)
 Lugol's Solution (2 - 30 ml bottles)
 Hydrochloric Acid (1 - 60 ml bottle)
Dextrose Powder (4 grams)
Manganese Dioxide (15 grams)
Starch Powder (3 grams)

Teacher Manual (1)
Student Instructions (1 Master)

Additional Required Materials:

Fresh Potato
Styrofoam Cups
Warm Water
Saliva Samples
Teaspoons

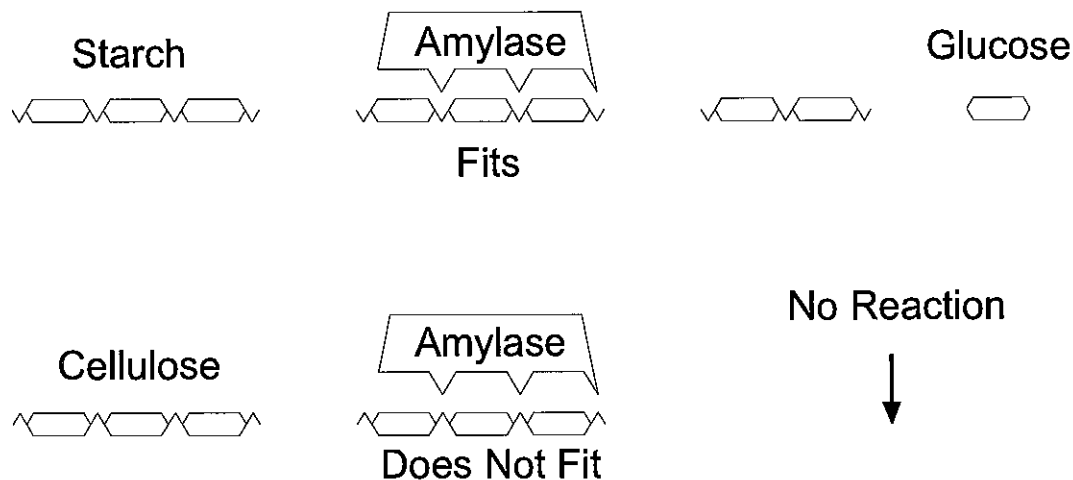
HOW POISONS WORK #1000 Teacher Manual**Introduction**

All life depends on a variety of organic catalysts called enzymes. Many Nobel Prize-winning studies in recent years have been on the structure and activity of these agents. The expanding field of genetic engineering is also largely concerned with their activity and structure. Many of the factors which influence enzyme activity have been areas of social concern as well. Many genetic disorders are characterized by particular enzyme malfunctions. Malnutrition deprives the body of essential building blocks and activators necessary for their production. Our environment has become polluted with a variety of chemicals that directly affect the action of enzymes. This kit focuses on the mechanism of enzyme activity, and the effect poisons have on that activity. This kit is designed to be used by secondary level biology students, and is probably best used along with class discussions of enzyme activity, metabolic disorders, or pollution. The topics of enzyme-catalyst, active site, substrate, and enzyme structure are introduced to explain the laboratory results.

Background Information

The modern theory of enzyme action is often called the "Lock and key" theory. Enzymes are proteins with complex molecular structures. Substrates, the enzyme's target molecules, must fit into the three-dimensional structure of the enzyme, much as a key fits into a lock. The substrate is held to or within the enzyme by loose chemical attractions, such as hydrogen bonds. Although enzymes are large molecules, only a small portion of the enzyme contacts the substrate. This region, the *active site*, consists of a group of amino acids from the protein chain of the enzyme. The various amino acids are oriented so that the substrate can first bind and then be converted to the desired products. The final product is then released by the enzyme, which returns to its original structure. Since the structure of the active site is so precise, each enzyme can act on only one type of substrate molecule. In the same way that only one key will fit a lock, of two similar substrate molecules only one has the precise fit required for reaction to occur. This property of enzymes is called their *specificity*.

The specificity of enzymes is illustrated schematically in Figure 1:

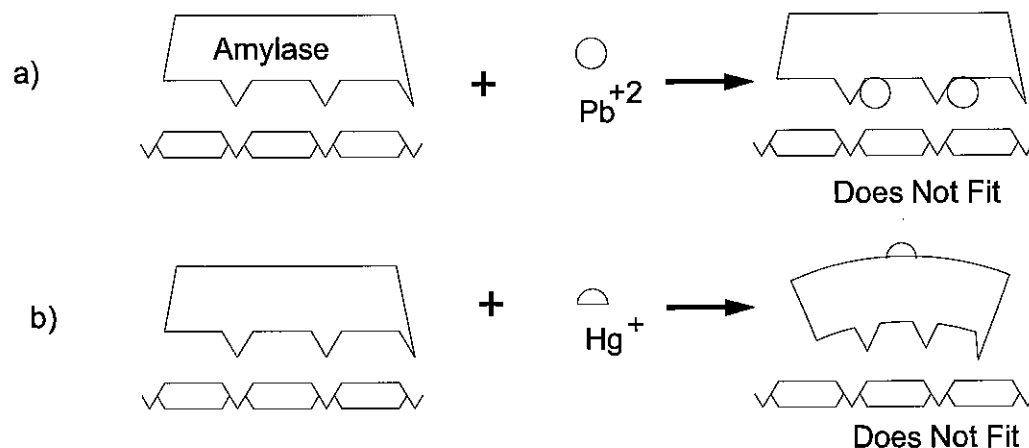


In this representation, the enzyme amylase catalyzes the removal of a glucose molecule from the end of a molecule of starch. The active site of the amylase molecule fits into the starch substrate, allowing reaction to occur. By contrast, a molecule of cellulose does not fit into the active site of amylase, and the reaction does not occur. Although cellulose and starch are both long chains of glucose molecules, they have different bonds between the sugar units. This slight difference is sufficient to make cellulose an unacceptable substrate for amylase. This is the reason that we cannot get energy by eating wood - our amylase enzymes will not catalyze the breakdown of the cellulose in wood.

Blocking Enzyme Activity - Poisons

Every one of the thousands of reactions that occur in our bodies depends on enzymes. If the activity of a particular enzyme is blocked in some way, the result may be serious. Many genetic diseases result from "faulty" enzymes that do not have the correct amino acids needed for a functional active site. The reaction that would have been catalyzed by the enzyme will not occur.

Even if the enzyme itself is normal, its activity may be blocked by various kinds of poisons. Poisons act on enzymes in two basic ways: by becoming locked into the active site of an enzyme, preventing it from acting on the correct substrate, or by changing the shape of the enzyme, thereby deactivating the active site. The first type of poison is typified by heavy metal ions, such as mercury, Hg^+ , or lead, Pb^{+2} . Considerable attention has been given recently to the harmful effects of mercury and lead pollution. Figure 2 shows schematically how lead might affect the action of the amylase enzyme. While not all heavy metals attach directly to the active site, Pb^{+2} is shown in the active site in Figure 2a to illustrate how the metal ion prevents the enzyme from attaching correctly. Figure 2b shows how a Hg^+ ion might attach to part of the enzyme other than the active site, indirectly affecting the active site.



Another example of the first type of poison is the arsenate ions, AsO_4^{3-} , which are chemically similar to phosphate ions. The phosphate ion, PO_4^{3-} , is involved in many important reactions involving enzymes. The arsenate ion, if present, will fill the active site normally occupied by a phosphate ion, and remain bonded there. The enzyme is prevented from acting on the phosphate ions.

Using The Kit In The Classroom

Objectives:

This kit allows the student to:

- Observe two enzymatically-catalyzed reactions, and measure the rates of reaction using appropriate techniques.
- Observe the effects of metal poisons on the two reactions, and to describe the decrease in reaction rate in terms of enzymes, substrate, active site, and catalysis.
- State at least one reason why lead and mercury are considered harmful pollutants in rivers, streams, etc.

The two enzymatic reactions to be studied are: 1) the breakdown of hydrogen peroxide into water and oxygen gas catalyzed by the enzyme *peroxidase* found in potatoes and 2) the breakdown of starch into glucose catalyzed by salivary *amylase*. The rate of the peroxidase/ H_2O_2 reaction will be measured by observing the rate of bubbling (oxygen production) in the solution. Differences in rates will be measured on a qualitative basis (faster or slower bubbling) rather than a quantitative measurement. Throughout the activity, you may wish to discuss possible means of taking quantitative measurements, and what those measurements might show about the system being studied. The rate of the amylase/starch reaction will be measured using the iodine-potassium iodide test for starch, also a qualitative test, and the Chemstrip test for the presence of glucose, a quantitative test. (Chemstrips may be used by diabetics to test for the presence of sugar in urine.)

Preparing The Materials

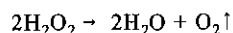
Make up the starch and glucose (dextrose) solutions by mixing the packets of starch and dextrose powder with 250ml of warm water (use distilled water, if possible). Use the pipets provided to dispense the solutions. Have a fresh potato available as well.

Guide To The Student Materials

Prior to beginning the labs, you should provide an introduction to both inorganic and organic catalysis of chemical reactions. Concentrate on enzyme activity, specificity, and the actions of poisons such as described in the Background Information section of this Teacher's Manual. Also, discuss the topic of inorganic vs. organic catalysis, giving the advantages and disadvantages of enzyme specificity and sensitivity as compared to inorganic catalyst's generality and insensitivity. Such an introduction will make the purposes of the various lab procedures clearer to the students.

Part A: Peroxidase/ H_2O_2 Reaction:

This section of the lab procedures deals with hydrogen peroxide's decomposition into water and oxygen in the following reaction:



The first step in this procedure establishes the fact that hydrogen peroxide does not decompose significantly under normal conditions. When the potato, which contains the enzyme *peroxidase*, is added, the rate of decomposition is greatly increased. (If you wish to demonstrate the oxygen production, set up a 250 ml Erlenmeyer flask with a large amount of peroxide solution and potato, and use the glowing splint test.) In the second step, the drastic reduction of oxygen production after the addition of mercury ions establishes mercury as a poison. Try to relate these results to the question of mercury pollution, and its effects on a wide variety of organisms.

Dispose of the waste water in a glass container and keep this container available for the disposal of Part B waste products.

Part B: Starch/Amylase

This section deals with the breakdown of starch into sugar (glucose) by an enzyme called amylase that is found in saliva. Diagrams of the reaction are in the Background Information section of the Teacher's Manual.

The first step in the procedure establishes the use of Lugol 's solution (iodine-potassium) as a test for starch.

The second step develops the use of Chemstrips as a test for the presence of sugar. Point out to the students that Chemstrips are used by diabetics to test for sugar in urine, and be sure that the students are able to use the color scale on the package correctly.

The third step establishes the following points:

- Saliva contains no starch or sugar (note: if the students have eaten just prior to this experiment, their saliva may test positive for sugar)
- The amount of starch in solution will decrease when saliva is added, as shown by the lighter color with the iodine solution
- Sugar is produced as the amylase acts on the starch

The fourth step shows that lead ion decreases the activity of amylase. Point out to the students that the solution of lead is quite dilute, yet it has a tremendous effect on the enzymes activity. Relate the effect of the lead to its alteration of the active site of the enzyme. Make sure that the students pour their waste materials into the glass container set aside for this purpose.

Try to develop the idea that there are thousands of enzymes besides amylase which will react in a similar way to the presence of lead. Lead poisoning is extremely serious, especially in large urban areas. In addition to the many deaths which lead-based paint has caused, children and adults have suffered serious brain, heart, and liver damage.

Part C: Organic And Inorganic Catalysts

The last set of experiments compares the action of enzymes with inorganic catalysts, demonstrating that poisons and pH changes do not affect inorganic catalysts as much as they do enzymes. The powdered manganese dioxide and the potato enzyme peroxidase will both catalyze the breakdown of hydrogen peroxide.

Step one establishes the fact that both the enzyme and the manganese dioxide both catalyze the breakdown of hydrogen peroxide. Step two demonstrates the organic catalyst's sensitivity and the inorganic catalyst's insensitivity to pH changes. The enzyme's sensitivity and the manganese dioxide's insensitivity to the poison lead ions is shown in Step 3. This should be related to the overall properties of enzymes as compared with

inorganic catalysts.

Disposal of the waste from these activities can be developed into a very interesting discussion of pollution, and man's effect on his environment. Hopefully, the students will point out the danger of pouring the lead solution down the drain. Develop the need to dispose of the waste in a way which will not introduce the poison to the water/waste system. The glass container filled with the waste from all classes should be disposed of according to state or local regulations. An additional disposal method involves precipitating out the soluble lead and mercury ions with a concentrated sodium chloride or sodium sulfide solution. By adding a small amount of either solution to the waste container, one can insure that the lead and mercury ions will not diffuse into the environment.

Topics For Discussion

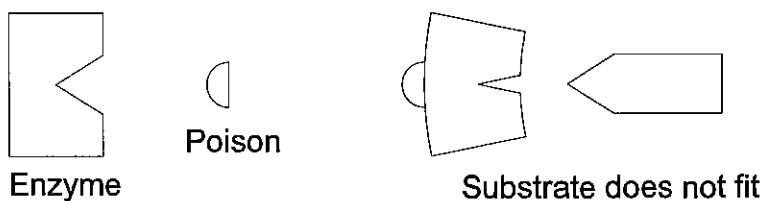
After completing the kit several areas may be discussed:

- 1 Discuss enzymes in more detail, concentrating on such features as their protein composition, structure, origin in the cell, sensitivity, etc.
- 2 Discuss various types of poisons and their target enzymes in organisms. Insecticides and nerve gases act on the enzyme cholinesterase in the nervous system. These poisons prevent the enzyme from breaking down acetylcholine, which in turn causes repeated nerve impulses, muscle spasms, and death. Relate the mechanism by which poisons act to other chemicals such as penicillin, which fills the active site of a certain bacterial enzyme.
- 3 Discuss the various sources of pollution. Lead is a common by-product of paint and petroleum industries, while mercury is often produced by paper manufacturing plants. Discuss problems of waste disposal as a large-scale industrial problem, and develop the need to find efficient, yet inexpensive, disposal procedures.

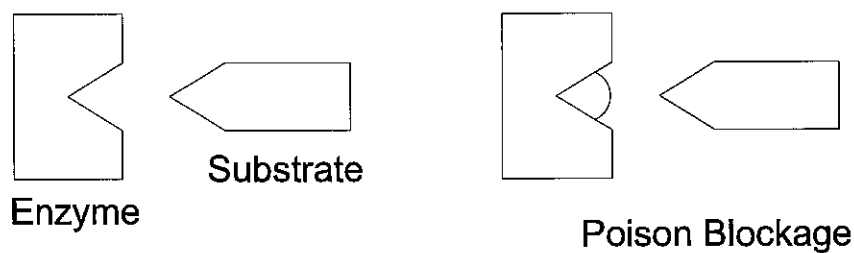
HOW POISONS WORK #1000 Student Instructions (annotated)

All life depends on the correct functioning of many different enzymes. Enzymes catalyze, or help along, specific reactions within the body. These enzymes are proteins that have complex structures with one specific area, the *active site*, that is designed to fit the *substrate* molecule exactly. The substrate, the molecule on which the enzyme will act, fits into the enzyme much like a key fits into a lock. Only one key will open a lock, and only one substrate will fit into an enzyme. The enzyme then catalyzes the reaction and releases the end product.

This kit is designed to show you how *poisons* work. Poisons can act on enzymes in two different ways. First, they can act by somehow changing the shape of the active site of the enzyme so that the substrate cannot fit properly.



Second, they may have a very similar structure to the substrate molecule and compete with the proper substrate for the enzyme active site.



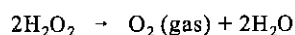
If the substrate cannot fit into the active site, then no reaction occurs, and the enzyme is deactivated. Mercury is a terrible poison. It is used to make batteries, paints, electrical switches and hundreds of products. Incineration of these items releases mercury into the atmosphere where it can remain for up to two years. At any one time the atmosphere contains 930 tons of this deadly material which slowly descends in rain and snow upon wildlife and

man.

Mercury has been linked to the deaths of panthers and loons in Florida and is suspected in the reproductive failures of eagles and minks of the Great Lakes Region. In the 1950's and early 1960's more than 100 people fell into comas and died in Minamata, Japan. The tragedy was linked to their diet of fish, heavily contaminated with mercury. Amounts of the toxin which are less than fatal result in slurred speech, mental dysfunction, and uncontrollable shaking.

PART A: Breakdown of Hydrogen Peroxide By Peroxidase

Many animal and plant materials contain an enzyme called *peroxidase*. This enzyme can catalyze the breakdown of hydrogen peroxide into water and oxygen:



This reaction can be observed by measuring the rate at which bubbles of oxygen are produced. Under normal conditions, the reaction is very slow, but in the presence of a material such as potato or liver, a high rate of reaction can be observed.

Place 2 ml (about two cm [1 in] high in the LabForm tube) of hydrogen peroxide solution in tube A of the LabForm. (*CAUTION: hydrogen peroxide can be harmful to the skin. so use careful technique during this part of the activity.*) Add a small piece of potato to the tube and record your observations.

After the potato is added, small bubbles rise from the surface of the potato; these continue at a relatively constant rate for several minutes.

Place 2 ml of peroxide solution in tube C of the LabForm. Add 2 drops of the mercury nitrate solution, then add another piece of potato to this tube. Record your results.

No bubbles or very few bubbles form in this case.

What effect does the mercury have on the activity of the peroxidase enzyme?

The mercury has apparently destroyed the ability of the peroxidase to breakdown the hydrogen peroxide. (Try to get the students to discuss the dangers of mercury)

Dispose of the reagents as your teacher instructs.

PART B: Starch/Salivary Amylase

Your saliva contains an enzyme called *amylase* which can break down starch into sugar. In this experiment, you will first measure the activity of amylase, and then test the effect of the poison, lead. Before beginning the experiment, saliva must be collected from either you or your partner in one of the small plastic cups provided.

Using a pipet, place 2 ml (a 2 ml point is marked on the pipet) of starch solution in tube A of the LabForm. With another pipet, place 2 ml of glucose solution in tube C. Add 2 drops of iodine solution to each of the tubes. Record any color changes below

Iodine plus starch turns bluish or dark purple. The glucose solution shows only the brownish yellow of the iodine solution

The color which you observed in tube A is a standard test for the presence of *starch*.

Using the starch pipet, place one drop of starch solution on the reagent end of a Chemstrip and, using the glucose (dextrose) pipet, one drop of glucose solution on another Chemstrip. Let each Chemstrip set for 1-2 minutes. Chemstrips will indicate the presence of glucose. Compare the colors with those on the case of the Chemstrips. Does starch contain any sugar which can be detected by the Chemstrip?

No (NOTE: if you heat the starch solution excessively in preparation, or let it sit for long periods of time, the test may be positive)

Using another pipet, place about 1 ml of saliva in tubes B, E, and G of the LabForm. Add 2 drops of the iodine solution to tube B and record the results.

Does saliva contain detectable starch?

No

Dip the reagent end of a Chemstrip into tube B containing the saliva/starch solution. Remove and let Chemstrip set for 1-2 minutes. Compare the Chemstrip with the color on the package. Does saliva contain any detectable sugar?

No (NOTE: if the students have just eaten, saliva may show detectable sugar)

Place 2 ml of starch solution in tubes E and G, and allow the mixtures to sit for 5 minutes. If it is available, immerse the LabForm in a styrofoam cup with warm water (Not hot) about 2/3 full. Warmth keeps the saliva at body temperature. After 5 minutes, add 2 drops of iodine solution in tube E and dip a Chemstrip into tube G. Let the Chemstrip set for 1-2 minutes. Record the results.

Tube E should show a less positive reaction for the starch, as compared with Tube A, and some sugar should be present in tube G.

What did the saliva do to the starch solution?

Saliva has, in some way, broken down the starch and created sugar. In fact the starch is digested into sugar molecules.

Clean out the LabForm, and place about 1 ml of saliva in tube A. Add 2 drops of the lead nitrate solution to the tube, then add 2 ml of starch solution. After 5 minutes, test the solution with a Chemstrip and record the results. What has the lead done to the activity of the amylase?

The Chemstrip should show no sugar produced. The lead prevented the saliva from performing its normal function.

Dispose of the reagents as your teacher instructs.

Until about 10 years ago, many houses, especially in cities, were being painted with lead-based paint. A particular danger is painting over existing paint. Even though non-leaded paint is now used, the old paint underneath still has lead pigments present. While the surface coat may be non-leaded, if it chips, the paint underneath may be exposed and may be eaten by children. (This paint has a mildly sweet taste). About 5% of children living in low-income urban areas suffer from some symptoms of lead poisoning.

Place 1 ml of saliva in Tube B the LabForm. Add 5 drops of hydrochloric acid to the saliva, then add 2 ml of starch solution. After 5 minutes, test the solution with a Chemstrip, and record the results.

The Chemstrip should show no glucose produced.

The saliva has been deactivated by the hydrochloric acid. Since enzymes are proteins, they are very sensitive to changes in the pH of their environment.

PART C: Organic Versus Inorganic Catalysts

Enzymes are *organic* molecules that catalyze specific chemical reactions in the body. To achieve this specificity (the ability to act on one type of molecule), enzymes are often very complex and fragile molecules. Other molecules can catalyze the same reactions as enzymes, and are much less susceptible to environmental changes and poisons. These *inorganic* molecules, though, lack specificity, and will act on very large numbers of different types of molecules.

We already know from Part A that an enzyme in potatoes, peroxidase, will catalyze the breakdown of hydrogen peroxide into water and oxygen. In this section, we will compare the effect of pH change and lead poisoning on this organic peroxidase enzyme and another, inorganic, catalyst called manganese dioxide.

Look back to Part A and record your results from putting potato pieces into hydrogen peroxide:

The hydrogen peroxide bubbled when the potato was put in it.

Get another small piece of potato (about 2 cm² is plenty), and cut it up into small pieces. Pour 1 ml (1 cm) of hydrogen peroxide into tubes A and C of the LabForm. Add 5 drops of hydrochloric acid to tube A, and some cut up potato. Record the results:

The hydrogen peroxide bubbled little or not at all.

To tube C of the LabForm, add 5 drops of lead nitrate, and then a small amount of potato. Record your results.

The hydrogen peroxide bubbled little or not at all.

Manganese dioxide is an example of one of these nonspecific inorganic catalysts. We have just used potatoes containing the enzyme peroxidase to catalyze the breakdown of hydrogen peroxide. Manganese dioxide catalyzes the same reaction. After rinsing it thoroughly, place 1 ml of hydrogen peroxide in tube A, C, and E of the LabForm. Add a sprinkle (about 1/8 teaspoon) of manganese dioxide to tube A and record the results.

The hydrogen peroxide bubbles (and turns steel grey).

Add 5 drops of hydrochloric acid to the hydrogen peroxide in tube C, then add a small amount of manganese dioxide to tube C. Record the results.

The hydrogen peroxide bubbles (and turns steel grey).

Add 5 drops of lead nitrate to tube E of the LabForm, and then the same amount of manganese dioxide as used before. Record the results.

The hydrogen peroxide bubbles (and turns steel grey).

Dispose of the lead nitrate solutions from the Labform as your teacher instructs.

Questions For Discussion

- 1 Some poisons, like mercury, can effect the activity of a large number of different enzymes. Others, like nerve gases, will effect only a particular enzyme. Explain.

The heavy metal poisons deactivate enzymes by binding on the enzyme molecules. They can bind on to many different protein molecules, including enzymes. Nerve gas and other poisons are similar in structure to the substrate molecules on which the enzyme is supposed to be acting. Since enzymes are specific in their activity, the poison is able to mimic the substrate for only particular enzymes.

- 2 Why do you think that living things rely on enzymes to catalyze reactions rather than the heartier inorganic catalysts, which are not effected by temperature, poisons, or acidity changes to any great extent.

Since life requires a precise balance of a wide variety of chemicals, it's important for an organism to have a mechanism for controlling each of the chemicals carefully. Enzymes provide this fine control because of their specificity, they act on specific molecules for which they have the correct active site structure. Inorganic catalysts tend to be much less specific in their activity. Also, the cell can control the amount of each enzyme which is produced, which provides yet another level of control.

- 3 Discuss the effects of a factory dumping large amounts of mercury into the river from which it draws water.

The algae and protozoa which live in the water would either die or accumulate high levels of mercury. Larger organisms which feed on these small ones would then suffer the loss of their food supply, would in turn accumulate high levels of mercury, or would die. Eventually humans might eat the toxic fish and become poisoned by this heavy metal, which causes mental and personality changes marked by depression, among other physical effects. The effects of water pollution can be felt many miles downstream from the point of pollution, and great distances inland as well. Just as the life of an individual organism is a sensitive balance, so is the life of a community of organisms.

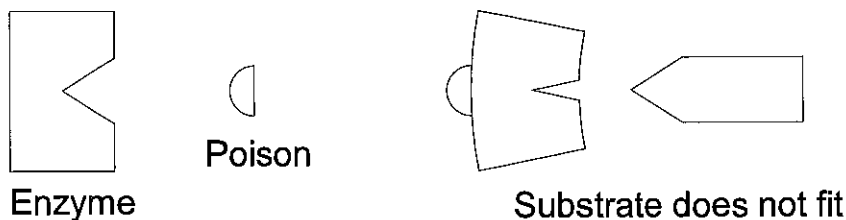
NOTE - In Minamata, a small fishing village in Japan, in the early 1950's, there was an outbreak of mercury poisoning. The disease showed in lesions of the central nervous system causing progressive weakening of the muscles, loss of vision, impairment of the cerebral functions, eventual paralysis, and sometimes death. Many of the areas sea birds also showed signs of mercury poisoning. This led to the discovery of high concentrations of methyl mercurials in fish and shellfish taken from the bay. The source of mercury was traced to the effluent from a factory.

- 4 If you have access to biology reference texts, discuss the means by which enzymes are produced in the cell.

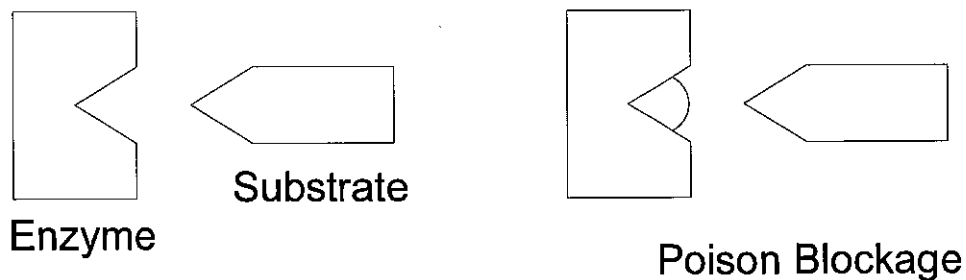
Genetic information is contained in molecules of DNA found in the nucleus of the cell. This genetic "code" is transferred to a similar molecule, RNA. The code is "read" on specialized structures called ribosomes. The code determines a specific sequence of amino acids, which are the components of proteins. In this way, the cell manufacture's the enzymes it needs.

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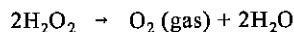
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Place 2 ml of peroxide solution in tube C of the LabForm. Add 2 drops of the mercury nitrate solution, then add another piece of potato to this tube. Record your results.

What effect does the mercury have on the activity of the peroxidase enzyme?

Dispose of the reagents as your teacher instructs.

PART B: Starch/Salivary Amylase

Your saliva contains an enzyme called *amylase* which can break down starch into sugar. In this experiment, you will first measure the activity of amylase, and then test the effect of the poison, lead. Before beginning the experiment, saliva must be collected from either you or your partner in one of the small plastic cups provided.

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Using another pipet, place about 1 ml of saliva in tubes B, E, and G of the LabForm. Add 2 drops of the iodine solution to tube B and record the results.

Does saliva contain detectable starch?

Dip the reagent end of a Chemstrip into tube B containing the saliva/starch solution. Remove the Chemstrip and let set 1-2 minutes. Compare the strip with the color on the package. Does saliva contain any detectable sugar?

Place 2 ml of starch solution in tubes E and G, and allow the mixtures to sit for 5 minutes. If it is available, immerse the LabForm in a styrofoam cup with warm water (Not hot) about 2/3 full. Warmth keeps the saliva at body temperature. After 5 minutes, add 2 drops of iodine solution in tube E and dip a Chemstrip into tube G. Remove strip and wait 1-2 minutes. Record the results.

What did the saliva do to the starch solution?

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To tube C of the LabForm, add 5 drops of lead nitrate, and then a small amount of potato. Record your results.

Manganese dioxide is an example of one of these nonspecific inorganic catalysts. We have just used potatoes containing the enzyme peroxidase to catalyze the breakdown of hydrogen peroxide. Manganese dioxide catalyzes the same reaction. After rinsing it thoroughly, place 1 ml of hydrogen peroxide in tube A, C, and E of the LabForm. Add a sprinkle (about 1/8 teaspoon) of manganese dioxide to tube A and record the results.

Add 5 drops of hydrochloric acid to the hydrogen peroxide in tube C, then add a small amount of manganese dioxide to tube C. Record the results.

Add 5 drops of lead nitrate to tube E of the LabForm, and then the same amount of manganese dioxide as used before. Record the results.

Questions For Discussion

- 1 Some poisons, like mercury, can effect the activity of a large number of different enzymes. Others, like nerve gases, will effect only a particular enzyme. Explain.
- 2 Why do you think that living things rely on enzymes to catalyze reactions rather than the heartier inorganic catalysts, which are not effected by temperature, poisons, or acidity changes to any great extent.
- 3 Discuss the effects of a factory dumping large amounts of mercury into the river from which it draws water.
- 4 If you have access to biology reference texts, discuss the means by which enzymes are produced in the cell.

SAFETY INSTRUCTIONS: IMPORTANT

NOTICE TO TEACHERS REGARDING LABORATORY REAGENTS

Perhaps the best general rule regarding the safe handling of laboratory chemicals is to treat all of them as being potentially dangerous. This means that none of them should be taken internally, and that any external contact should be washed thoroughly. In fact, most of the chemicals provided in The Science Source kits are diluted enough that they are not hazardous. The following lists indicate appropriate antidotes for the hazardous chemicals. Check this list for the chemicals provided in the kit:

- I. **Concentrated Acids & Bases** - **Do not** induce vomiting, dilute with water, then milk or egg white, call a physician immediately.
 1. 25 % Acetic Acid
 2. 3M Hydrochloric Acid
 3. Concentrated Sulfuric Acid

- II. **Dilute Acids & Bases** - **Dilute** with water, then milk.
 1. 1 M, 0.5M, 0.1 M Hydrochloric Acid
 2. Oxalic Acid
 3. Sodium Hydroxide
 4. Ammonium Hydroxide

- III. **Miscellaneous Chemicals** - Dilute immediately with water. Induce vomiting with warm salt water, or warm baking soda solution.
 1. Ammonium Chloride
 2. Ammonium Oxalate
 3. Barium Chloride
 4. Biuret Reagent
 5. Bromthymol Blue
 6. Calcium Chloride
 7. Ethanol (Denatured Alcohol)
 8. Ferric Ammonium Sulfate
 9. Hydrogen Peroxide
 10. Janus Green B
 11. Lead Nitrate
 12. Lugol's Solution
 13. Magnesium Reagent (Titan Yellow, Alcohol)
 14. Mercuric Nitrate
 15. Methylene Blue
 16. Ninhydrin
 17. Phosphorus Reagent (Ammonium Molybdate, Nitric Acid)
 18. Potassium Ferricyanide
 19. Potassium Permanganate
 20. Silver Nitrate
 21. Sodium Carbonate
 22. Sodium Thiosulfate
 23. Sudan IV

- IV. **Organic Solvents** - **Do not** induce vomiting. Dilute with water and milk. Call a physician immediately.
 - Isopropyl Alcohol