Cell Function Exercise

Living matter exhibits several properties that distinguish it from nonliving matter, such as irritability (the capacity to respond to stimuli), metabolic transfer of energy, movement, biosynthesis of cellular constituents and their maintenance and repair, exchange with the environment, and, especially, reproduction. Several characteristics of living cells and tissues can be illustrated in the laboratory by simple experiments that you can perform, and some by demonstrations set up by the instructor.

EXERCISE A:
Movement of Materials across Cell Membranes
Brownian movement
Diffusion and osmosis
Carrier-mediated transport

EXERCISE B:
Action of Enzymes
Part 1: Effect of temperature on enzyme activity
Part 2: Effect of pH on enzyme activity

EXERCISE A

Movement of Materials across Cell Membranes

One of several ways that materials may cross membranes is by **diffusion,** the random movement of molecules and ions from regions of higher concentration to regions of lower concentration. This happens because environmental heat imparts energy to molecules and ions, causing them to bounce against each other. Thus if we drop some dye into a beaker of water, it will diffuse slowly through the water until the dye molecules are equally distributed throughout.

Osmosis is a special case of diffusion of a solvent (which is always water in living systems) through a selectively permeable membrane from a dilute solution to a more concentrated solution. Both simple diffusion and osmosis result from kinetic activity of molecules and are therefore considered to be passive processes.

Important as simple diffusion and osmosis are, they do not account for all movement of materials through living membranes. Many materials move *against* concentration gradients. Such materials are moved either by **carrier-mediated transport** involving membrane protein carriers or by **endocytosis** (ingestion of fluids or solids into cells).

Brownian Movement

The constant motion of molecules and ions mentioned previously results from kinetic energy imparted to them by heat. The higher the temperature and the smaller the molecule or ion, the more rapid the molecular motion, whereas at absolute zero, molecular motion stops altogether.

Although we cannot see high-speed bouncing of individual molecules in solution, we can see the *results* of their motion, as millions of water molecules collide with particles suspended in the water. The phenomenon is called **brownian movement.**

1. **Thoroughly crush a bit of living tissue, such as a piece of a worm or insect. This may be done with a mortar and pestle or a tissue grinder or even by mashing the living fragment on a microscope slide with a solid glass rod that has been flame polished on one end. Add a very small amount of water to the tissue if necessary to assist grinding. After removing larger pieces of tissue, transfer some of the creamy residue to a drop of water on a clean slide. A toothpick is handy for this purpose. Cover with a cover glass and examine with high power, reducing the light as necessary. When all water currents have ceased, look for the smallest freely suspended particles you can see and watch their movement.**

Even the smallest visible particles are far larger than molecules. Their movement is the result of recoil when they are struck by rapidly moving water molecules.

When in 1827 the English botanist Robert Brown first described the movement that now bears his name,

he attributed the motion to minute living organisms. Brown knew nothing of the kinetic theory of energy that was developed some years later, so his conclusion was reasonable. Nevertheless, had Brown performed the following simple experiment with nonliving particles, might he have reached a different conclusion?

2. **On a clean slide place a drop of water containing a suspension of powdered carmine or India ink and add a coverslip. Again try to follow a single particle.**

What do you conclude about the presence of brownian movement in living and nonliving material? Answer the questions on page 4. The movement will continue indefinitely or for as long as you can keep the water from evaporating.

Diffusion and Osmosis

Diffusion

Free diffusion is the result of constant random motion of all molecules, witnessed in the preceding exercise. It occurs rapidly in gases, more slowly in liquids, and extremely slowly in solids. Diffusion is temperature dependent, occurring more rapidly as the temperature rises. It also is dependent on molecular size, and small molecules or ions diffuse more rapidly than large ones.

Effect of Temperature on Rates of Diffusion

Your instructor will set up three beakers containing water of different temperatures. The first beaker is placed in an ice bath and contains cold water (about 4° C). The second beaker is at room temperature (about 22° C), and the third beaker contains hot water (about 85° C).

The instructor will drop a tiny crystal of potassium permanganate into each beaker. Begin your observations immediately and record the actual temperatures and diffusion results in the table on p. 4.

Effect of Molecular Size on Rates of Diffusion

In this experiment you will compare the diffusion rates of ionic groups with different molecular weights through a gel-like medium. The instructor will provide you with a Petri dish containing agar, which is a gelatin-like substance extracted from seaweed (red algae).

Carefully **punch four holes in the agar with a no. 5 cork borer at the 12, 3, 6, and 9 o'clock positions and remove the agar plugs. Now add several drops of the following solutions as directed, filling the holes uniformly and not allowing them to overflow: silver nitrate ($AgNO_3$) in the 12 o'clock hole, potassium ferricyanide ($K_3Fe(CN)_6$) in the 3 o'clock hole, potassium bromide (KBr) in the 6 o'clock hole, and sodium chloride (NaCl) in the 9 o'clock hole. The ion groups will diffuse radially until they meet and form lines of precipitation. Measure the distance in millimeters that each ion group has diffused and record in the table on p. 4.**

Osmosis

Osmotic pressure is one of the forces that determine the movement of water into and out of cells. Osmosis is the movement of solvent molecules (water) through a differentially permeable membrane. A differentially permeable membrane permits solvent molecules to pass through it freely but restricts the movement of solute molecules. For example, if water (the solvent) and a sugar solution (the solute) are separated by such a membrane, water molecules pass by diffusion through the membrane but sugar molecules cannot pass through. The result is a net movement of water into the sugar solution.

Let us examine more closely what is happening. Suppose that a 10% sucrose solution is in a bag composed of a differentially permeable membrane, and that the bag is placed in a container of distilled water. Knowing the molecular weights of water (18) and the sugar solution (342), it is possible to calculate that there are approximately 169 water molecules in the sugar solution for every molecule of sugar. An equal volume of distilled water outside contains approximately 170 molecules of water. Because of molecular motion, the membrane will be bombarded with molecules from both directions. Out of 170 hits on the inside, 169 are from water molecules and 1 is from a sugar molecule; however, on the outside, all 170 hits are by water molecules. Because the membrane is permeable to water but not to sugar, slightly more water molecules enter the sugar solution than leave it. The result is a net flow of water inward; the sugar solution volume will increase as the water volume outside decreases. Actually, passage of water inward is favored even more than our example suggests, because some of the water in the sugar solution becomes complexed with sugar molecules, leaving less free water to diffuse outward. Furthermore, free water molecules cannot pass out as readily as they can pass in because of interference by sugar molecules inside.

If we then fit a vertical tube to the bag of sugar solution, fluid will rise in the tube as water enters the solution across the membrane. This will continue until the hydrostatic pressure developed by the rise of fluid in the tube equals the osmotic pressure of the sugar solution. We can consider osmosis to be nothing more than the tendency of water molecules to move from a region of higher water concentration (distilled water in our example) to a region of lower water concentration (the sugar solution). Osmotic pressure is therefore a measure of the "escaping tendency" of water. Note that a differentially permeable membrane is essential for osmosis to occur. If the membrane is permeable *only* to water, it is said to be **semipermeable.** If the membrane allows one material

(e.g., sugar or salt) to pass through more readily than another, it is said to be **selectively permeable.** Both kinds of membranes are present in living systems.

Measuring the Rate of Osmosis

An osmometer similar to that shown in Figure 1 has been prepared. It consists of a tubular piece of unvarnished cellophane tied to a rubber stopper that is fitted to a long piece of glass tube. The cellophane tubing is filled with a concentrated sucrose solution.

Gently lower the osmometer into a jar of water until the top of the rubber stopper is level with the water surface. Check the level of the sugar solution. If it is below the level of the top of the rubber stopper, wait until it rises to that level, which is your zero point. Mark the zero point with a wax pencil. At intervals of 10 to 15 minutes record on p. 5 both the time and the height of solution in the glass tube in millimeters. Continue until the end of the period. Consult your instructor if the fluid column fails to rise or if it falls (osmometers occasionally develop leaks). Complete the report on p. 5.

Demonstrating Osmosis with Animal Membranes

Red blood cells serve as an excellent model system to demonstrate the osmotic properties of the plasma membrane. Red blood cells, like other body cells, are in osmotic equilibrium with the blood plasma; that is, the solute concentration inside the cells is about the same as the solute concentration outside. If a cell is placed in a **hypotonic** (literally "below tension") medium such as distilled water, water enters the cell by osmosis and causes it to swell and burst. The cell has then **hemolyzed.** Conversely, if a red blood cell is placed in a **hypertonic** ("above tension") medium, it shrinks (water is drawn out by osmosis) and the cell margins appear crenate, or scalloped.

For this experiment you will need three clean slides, coverslips, mammalian whole blood, and salt solutions.

On slide A place a drop of physiological saline solution (0.9% NaCl) and a drop of blood. Cover with a cover glass and examine under high power of a compound microscope. This solution is **isotonic** ("equal tension") and should cause no change in cell shape. Sketch the blood cells on p. 5. The appearance of these cells will serve as a control for comparison with the next two preparations. On slide B mix a drop of distilled water and a drop of blood. Cover with a cover glass and examine at once. What happens to the cells? Sketch on p. 5. On slide C mix a drop of blood with a drop of 5% NaCl and cover with a cover glass. Examine and sketch. Wait a few minutes, then examine and sketch again. Has the appearance altered? Complete the report on pp. 5–7.

Carrier-Mediated Transport

The experiments you have done thus far might suggest that the plasma membrane is impermeable to everything but water. Obviously, this is not true because it is essential that many materials, such as nutrients and materials for growth, must enter the cell, and wastes must leave it. Although some solutes do enter and leave cells by passive diffusion, most materials of biological significance are *carried* across the plasma membrane by special transport mechanisms built into the structure of the membrane. This is called **carrier-mediated transport** because protein carriers in the membrane "mediate," or assist, the transport of important molecules.

We can recognize two kinds of mediated transport: **facilitated diffusion,** in which the protein carrier helps a molecule move across an otherwise impermeable membrane, and **active transport,** in which energy is supplied to the carrier system to transport molecules across the membrane. Unlike facilitated diffusion, which does not require energy and can only move molecules *down* a concentration gradient, active transport requires metabolic energy and can move molecules in an *uphill* direction; that is, against a concentration gradient.

In this exercise, you will observe the **active transport** of a dye across the isolated kidney tubules of goldfish. As the dye accumulates in the tubule, its color becomes more intense than in the surrounding medium.

The instructor will prepare a goldfish by sectioning the spinal cord. Open the body cavity, push aside the gut, and quickly dissect out the kidney (brown tissue lying along the dorsal side of the body cavity). Place it in a large Petri dish containing the basic saline medium provided. Cut the kidney into small pieces with scissors. Then tease the pieces into *finely divided fragments* with a teasing needle or the tip of a hypodermic needle. No fragments should be left larger than 1 mm in diameter. With a pipette, transfer two or three small tubule fragments into the depression of a depression slide, and then cover the tubules with fresh basic saline medium. Examine the tubules with low power of a compound microscope or high power of a dissecting microscope. You should be able to see the tubules protruding from the tissue fragments. Blot or pipette the saline medium from the depression and replace with a test medium containing chlorophenol red solution.

Examine the tissue at intervals of 5, 10, 20, 30, and 45 minutes. You should see some dye beginning to accumulate within the tubules within 5 or 10 minutes. The dye will accumulate only in certain parts of the tubules because in goldfish only about 10% of the tubule is capable of transporting the dye. Complete the report on p. 7.

LAB REPORT

Name _____

Date _____

Section _____

Movement of Materials across Cell Membranes

Brownian Movement

What evidence of molecular movement did you see? _____

What evidence exists that brownian movement is not restricted to living material? _____

What causes brownian movement? _____

Under what conditions would all movement cease? _____

Diffusion

1. Record of permanganate diffusion.

	Extent of diffusion after				
Water temperature	1 min	5 min	10 min	20 min	60 min
(cold)					
(room)					
(hot)					

Summarize and explain the results recorded in the table above. _____

2. Record of diffusion of salt solutions in agar.

Position of salt	Salt	Ion/molecular weight	Extent of diffusion
12 o'clock	Silver nitrate ($AgNO_3$)	NO_3/112	
3 o'clock	Potassium ferricyanide ($K_3Fe(CN)_6$)	$Fe(CN)_6$/212	
6 o'clock	Potassium bromide (KBr)	Br/80	
9 o'clock	Sodium chloride (NaCl)	Cl/35	

From your results, what relationship exists between the rate of diffusion of the ion and its molecular weight?

Osmosis: Use of a Simple Osmometer (p. 3)

Record the time and height of the column in millimeters for each reading of the osmometer.

Time	Height	Time	Height	Time	Height

Plot on graph paper (p. 6) the height of the column on the ordinate (Y-axis) and the time in minutes on the abscissa (X-axis). Plan your plot to avoid crowding. Label and title the graph and attach to your laboratory report.

Describe the shape of the curve on your graph and explain its significance.

Explain the forces involved in the rise of the solution in the tube.

What is the difference between simple diffusion and osmosis?

Osmosis in Red Blood Cells

Sketch the blood cells as you saw them in these solutions:

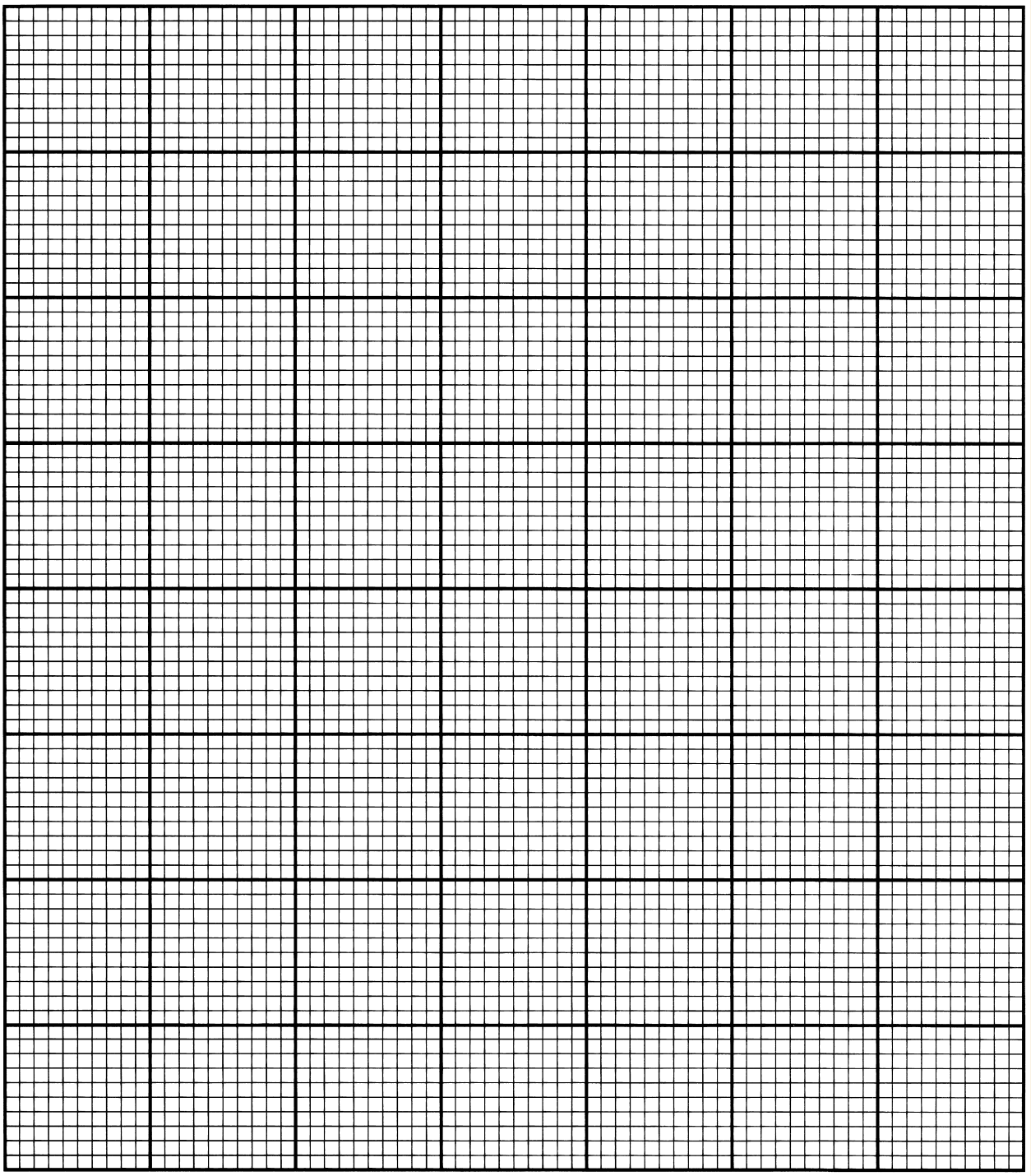
(1) Isotonic	(2) Hypotonic	(3) Hypertonic

LAB REPORT

Movement of Materials across Cell Membranes—cont'd

Name _____

Date _____ **Section** _____



From these results what can you conclude about the permeability properties of the red blood cell membrane to water and salt?

Carrier-Mediated Transport

Record dye concentrations in the goldfish kidney tubule using an arbitrary scale from minimal (0 or +) to maximal (+ + + +).

Dye concentration in tubule at					
0 min	5 min	10 min	20 min	30 min	45 min

Define active transport and explain how it can be distinguished from facilitated diffusion.

Why is dye accumulation in the goldfish tubules evidence of active transport?

EXERCISE B

Action of Enzymes

Enzymes are a special class of proteins that catalyze nearly all chemical reaction in cells. If enzymes were absent, cellular reactions would proceed at a negligible rate. Enzymes speed reactions by reducing the amount of activation energy that substrates require to have strong chemical bonds disrupted. The enzyme combines with the substrate to form a precisely aligned enzyme-substrate complex. The substrate is split after passing through one or more intermediate steps, which require much less energy than would a single-step reaction. The shape of the enzyme molecule is crucial to the reaction because an exact molecular fit between substrate and enzyme is required. Thus with few exceptions an enzyme will catalyze only one reaction and no other. Enzymes also help two smaller substrates form a larger product.

Although enzymes permit chemical reactions to proceed at the relatively low temperatures of animal bodies, they are sensitive to temperature, working faster at higher temperatures and more slowly at lower temperatures. Enzymes are also sensitive to pH (hydrogen ion concentration). Most cellular enzymes operate best at the near-neutral pH of the cellular environment, but many of the digestive enzymes work well in slightly alkaline (salivary and intestinal enzymes) or strongly acid (stomach enzymes) conditions.

In this experiment the action of the enzyme α-amylase, which is present in the saliva and pancreatic juice of many vertebrates, will be studied. Amylase promotes the hydrolysis of starch to maltose, a disaccharide composed of two glucose units. Maltose, like glucose, is a reducing sugar and will give a positive test result with Benedict's solution. In this reaction, the copper hydroxide ($Cu(OH)_2$) in the Benedict's solution is reduced (i.e., the oxidation number is reduced) by the aldehyde group of the sugar, forming a red precipitate of cuprous oxide (Cu_2O). The sugar becomes more oxidized in character.

The class will be divided into two groups, with one half studying the effects of temperature on enzyme activity (Part 1), and the other half studying the effect of pH on enzyme activity (Part 2). At the end of the period experimental data will be exchanged between the two groups. Laboratory reports should be prepared using data from both Parts 1 and 2.

Preparation of Dilute Amylase for Parts 1 and 2

Each student pair, whether assigned to the temperature experiment (Part 1) or to the pH experiment (Part 2), will first prepare a diluted amylase solution. Place 1.0 ml of 0.25% amylase in a test tube, and add 12 ml of water. This is your working diluted amylase solution, used in all of the experiments that follow.

Part 1: Effect of Temperature on Enzyme Activity

In this four-part experiment, you will examine the effect of temperature on the *rate* of enzymatic activity. Since amylase breaks down starch (the substrate) to maltose (the product), a convenient assay of enzymatic activity is to measure the time required for all of a given quantity of starch to disappear from solution. It is important to follow the preparations and testing procedures *carefully.*

Preparations

Prepare for the test by labeling four test tubes *A1* through *A4*. In tubes A1, A2, and A3 place 2 ml of the diluted starch solution. Now add 2 ml of pH 7.0 buffer to each tube. Buffers are mixtures of substances that interact so that the pH of the mixture remains constant even though small quantities of acidic or basic substances are added. Tube A1 is to be left at room temperature. Place tube A2 in a beaker of hot tap water. Let it stand, keeping the water in the beaker quite warm to the touch by replacing it with hot tap water from time to time. The temperature of the water bath should be quite warm at all times but not scalding hot. Place tube A3 in a beaker of ice water, and let it stand. Some unmelted ice must always be present in the beaker. To tube A4, which is empty, add 2 ml of the diluted amylase solution, put the tube in a boiling-water bath, and leave it until it is ready for testing. Do not allow the tube or beaker to boil dry; if some water has evaporated from the tube, add water as required to maintain the original volume.

Testing for Amylase Activity

Prepare for starch tests by placing one drop of iodine-potassium iodide (I-KI) solution in each depression of your porcelain test plate. To tube A1 (room temperature) add 2 ml of unheated diluted amylase. Agitate to mix thoroughly, and immediately test for the presence of starch by adding a drop of the I-KI in one depression. The development of a deep blue or black color indicates starch is present. Continue to test for starch in this way at short intervals, initially about 30 seconds, keeping track of the elapsed time. Use a different depression for each test. As the reaction proceeds, the dark color will be less apparent because the starch is being broken down. The point at which there is no longer any starch left is called the end point. This point is reached when the I-KI does not change color. Record the time required to reach the end point.

The appearance of maltose is another indicator of enzymatic activity. To the mixture remaining in the test tube, add about half as much Benedict's solution as the volume of remaining mixture. Test for the presence of reducing sugar (maltose) by placing the tube in a beaker of boiling water. If maltose or other reducing sugar is present, the deep blue color of copper hydroxide will change to an orange-red as cupric hydroxide is reduced to red cuprous oxide. Watch the tube until no further color change occurs—at least 5 minutes.

Note that you have tested for both the *disappearance* of substrate (starch test) and the *appearance* of product (Benedict's test).

Wash your porcelain test plate and proceed with tube A2 exactly as described for tube A1. Keep the tube in the warm water bath while the starch tests are being performed. It will be necessary to test for starch at short intervals because the reaction with tube A2 should occur quite rapidly. Record the time required to reach the end point and the results of the test for reducing sugar.

Wash the test plate and proceed with tube A3 exactly as with tubes A1 and A2. Keep the tube in the ice bath while the tests are being made. Much longer intervals may be used in testing the contents of this tube. Record the time required to reach the end point and the results of the test for reducing sugar.

You are ready for the final test with test tube A4. This test differs from the preceding three because you will use the amylase that has been boiled. Remove the tube from the boiling bath, and if necessary restore the original volume with tap water. Add an equal volume of diluted starch solution and 2 ml of pH 6.8 buffer. The resulting mixture is similar to those of the previous tests, except that *boiled* amylase is present. The tube should be kept in the test tube rack while the tests are performed. Proceed as in the previous tests. Perform the starch tests at intervals until near the end of the period, at which time the solution remaining in the test tube should be tested for the presence of reducing sugar. Record the results.

Part 2: Effect of pH on Enzyme Activity

In this experiment, you will examine the effect of pH on the rate of enzymatic activity of amylase. Since amylase hydrolyzes starch (substrate) to maltose (product), a convenient assay of enzyme activity is to measure the time required for all of a given quantity of starch to disappear from the solution. It is important to follow the preparations and testing procedures *carefully.*

Preparations

Place 2 ml of diluted starch solution in each of three test tubes. Be sure to shake the bottle containing the stock solution of starch so that the starch is thoroughly mixed. Add 2 ml of pH 7.0 buffer to the first tube, and mark this tube B1. To a second tube add 2 ml of pH 8.0 buffer, and mark this tube B2. To a third tube add 2 ml of pH 3.4 buffer, and mark this tube B3. Agitate all tubes to mix thoroughly. Keep the tubes in the test tube rack at room temperature during the tests.

Testing for Amylase Activity

Prepare to test for starch by placing one drop of I-KI solution into each depression of the porcelain test plate. To tube B1 add 2 ml of diluted 0.25% amylase and agitate to mix thoroughly. Immediately place one drop into a depression with I-KI solution. The development of a deep blue or black color indicates starch is present. Continue to test for starch at short intervals, initially about 30 seconds, keeping track of the elapsed time. Use a different depression for each test. As the reaction proceeds the color will be less apparent. The end point has been reached when no change in color can be detected. Record the time required to reach the end point.

To be sure that this enzymatic reaction has indeed produced maltose as a product, you will test for its presence with the Benedict's test for reducing sugar. To the mixture remaining in the test tube, add one half as much Benedict's solution as volume of remaining mixture. Test for the presence of reducing sugar (maltose) by placing the tube in a beaker of boiling water. If maltose or other reducing sugar is present, the deep blue color of copper hydroxide will change to an orange-red as cupric hydroxide is reduced to red cuprous oxide. Watch the tube until no further color change occurs—at least 5 minutes.

Note that you have tested for both the *disappearance* of substrate (starch test) and for the *appearance* of product (Benedict's test).

To tube B2 add 2 ml of diluted amylase and test for the disappearance of starch as just described. Record the end point time. Test the remaining mixture in tube B2 for the presence of reducing sugar with the Benedict's test.

To tube B3 add 2 ml of diluted amylase and test for the disappearance of starch as before. Record the end point time. Test the remaining mixture in tube B3 for the presence of reducing sugar with the Benedict's test.

Written Report

Summarize your observations on pp. 10–11.

LAB REPORT

Name _____

Date _____

Section _____

Action of Enzymes

Action of Enzymes

1. **Effect of temperature on enzyme activity**

Summarize the results of the tests for amylase activity with the I-KI solution for the four different temperature conditions. _____

Summarize the results of the tests for the appearance of product (maltose) with the Benedict's solution for the four different temperature conditions. _____

Why cannot the Benedict's test alone be used as an assay of enzymatic rate? _____

State your conclusions regarding the effect of temperature on an enzyme-catalyzed reaction. _____

2. **Effect of pH on enzyme activity**

Summarize the results of the tests for amylase activity with the I-KI solution for the three different pH conditions.

Summarize the results of the tests for the appearance of product (maltose) with the Benedict's solution for the three different pH conditions.

What can you conclude concerning the effect of hydrogen ion concentration on the "active" site of an enzyme?