A fourth trial was also performed when more enzymes were added. First, two enzymes catalyzed the reaction and then a third person was added afterwards. The rate of reaction drastically increased with the addition of enzymes. In the trial with three enzymes, all of the toothpicks (40) were broken in 30 seconds and the enzymes were free of substrates. This shows that the addition of enzymes increases the rate of reaction until there are no substrates left or all enzymes are saturated with substrates.

The experiment provided students with a hands-on method of learning how different conditions and factors affect enzyme activity. By acting as enzymes, students were able to think about the factors that made it difficult to break toothpicks and learn how enzymes work.

AP Bio Lab #4 - Diffusion and Osmosis

Lab #4 22/9/14

Introduction

Plasma and organelle membranes are selectively permeable in cells, meaning some substances cross easier than others. They consist of a phospholipid bilayer and embedded proteins. The hydrophobic tendency of the bilayer limits water movement. Water can pass through the membrane slowly by osmosis or with the help of aquaporins, which are specialized channel proteins. These aquaporins allow water to move across the membrane much faster.

Diffusion is a simple form of movement that does not require energy input, like osmosis. The movement of solutes from an area of high concentration to one of low concentration is called diffusion. However, the movement of solutes from low to high concentration does require energy input (in the form of ATP accompanied by protein carriers called pumps) and is called active transport.

Osmosis is a form of diffusion, where water moves down its concentration gradient. This means that water moves from areas of high free water concentration and low solute concentration to areas of low free water concentration and high solute concentration (since H_2O molecules surround solute molecules).

Isotonic solutions have equal water potentials, which means that water and solute cross the cellular membrane at the same rate. However, when the surrounding solution is hypertonic to the cell, water will move out of the cell and into the solution, because there is higher solute concentration and lower water potential. When the surrounding solution is hypotonic, the solute concentration is lower, the water potential is higher, and water will move into the cell.

In animal cells, water moving out will cause the cell to shrivel and shrink, and water moving in will cause swelling or bursting, due to the solute concentrations inside and outside the cells.

In plant cells, there is also turgor pressure, which resists water movement into the cell, preventing it from bursting and creating pressure. If water continues to leave the cell, it could plasmolyze, when the plasma membrane shrinks away from the cell wall.

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Introduction continued:

Water potential is abbreviated with psi (Ψ) and measures the tendency of water to move from one compartment to another. It can be calculated with this formula:

$$\Psi = \Psi_p + \Psi_s$$

Water potential = Pressure Potential + Solute Potential

The solute potential (Ψ_s) is dependent on solute concentration and can also be called osmotic potential.
Water potential predicts which way water diffuses through plant tissues, when water moves from an area of higher water potential to one of lower water potential.

Pure water in a beaker has a water potential of zero, because solute and pressure potentials are zero, however the addition of solute lowers solute potential (also lowering water potential) and an increase in positive pressure raises the pressure potential (also raising water potential).

Solute potential can be calculated with this formula: $\Psi_s = -iCRT$, where i = ionization constant, C = molar concentration, R = pressure constant ($R = 0.0831$ liter bars/mole-K), and T = temperature in Kelvin (273°+C°).

A bar is a measure of pressure. Atmospheric pressure means that $\Psi_p = 0$.

If a cell is surrounded by pure water, water will move into the cell because water potential is lower due to the solutes in the cytoplasm. This causes swelling and an increase in turgor pressure. Eventually water potential of cell and pure water become equal, because the positive turgor pressure opposes negative solute potential. Then dynamic equilibrium is reached.

If solute is added to the water around the cell, water will leave the cell. Eventually the water potential will be equal inside and outside the cell, if enough solute is added. This does not mean that solute concentrations inside and outside the cell are equal, because the cell is influenced by both turgor and solute pressure. If even more solute is added, water will leave the cell, meaning the cell will also lose turgor. Eventually the cell could plasmolyze.

- The solute potential of a 0.1 M NaCl solution at 25°C is −4.95 bars. If the NaCl concentration inside the cell is 0.15 M, the water will move into the cell from an area of higher free water concentration to an area of lower free water concentration.
- If there is no net diffusion between the cell and solute, turgor pressure must equal zero.

In this lab, students will investigate the relationship among surface area, volume, and rate of diffusion. The purpose is to connect the concepts of diffusion and osmosis to cell structure and function, while working collaboratively to design experiments and analyze results. Students should analyze the collected data and predict molecular movement through cellular membranes. Students will learn about osmosis, diffusion, and water potential in cells.

Hypothesis

- Procedure 1: If the three potato cells are submerged in iodine solution and left there for 20 minutes, then the nutrients should diffuse farthest proportionally in the smallest potato cube.
- Procedure 2: If one solution is put in the model cell (dialysis tubing) and one is put in the beaker and the cell and the beaker is left alone for 30 minutes, water should diffuse from the solution with higher free water concentration to the solution with lower free water concentration.
- Procedure 3: If a ~~set~~ single leaf blade from red onion becomes exposed to a solution from Procedure 2, water should either enter or leave the cells, depending on the solution. Turgor pressure should increase if the solute concentration on the inside of the cell is higher than on the outside.

Materials

The materials used include:

- Procedure 1
  - ruler
  - calculater
  - scalpel
  - potato pieces
  - paper towels
  - iodine solution
- Procedure 2
  - Tap/distilled water
  - 1M sucrose
  - 1M NaCl
  - 1M glucose
  - 5% ovalbumin
  - cups and balances
- Procedure 3
  - red onion leaf
  - light microscope
  - slides
  - chosen solution from Procedure 2

Procedure

- Procedure 1
  1. Cut three potato cubes using a scalpel into 0.5, 1, and 2cm cells.
  2. Obtain teacher sign-off before continuing.
  3. Put potato cubes into iodine solution for 20 minutes.
  4. Calculate surface area, volume, and ratio for each cube while waiting.
  5. Remove cubes and place on paper towels.
  6. Cut each cell in half and observe the inside.
  7. Draw a detailed drawing of the pattern of iodine throughout the potato.
- Procedure 2
  1. Choose up to four pairs of different solutions. One solution from each pair will be in the model cell of dialysis tubing, and one will be outside the cell in the cup. ~~Pr~~
  2. Predict whether water will diffuse in or out of the model cell.
  3. Label the cups with the solution that is inside the cell and inside the cup.
  4. Make dialysis tubing cells by tying a knot in one end of five pieces of tubing. Fill each "cell" with 10ml of solution & knot other end. Leave enough space for diffusion! Don't forget to make a control with water.
  5. Weigh each cell & record initial weight. Place into cup with other solution.
  6. Wait 30 minutes and record final weight afterwards.
  7. Calculate the percent change in weight: (final-initial)/initial · 100
  8. Record results.
- Procedure 3
  1. Look at a single leaf blade under light microscope. Observe the cells and determine how a solution might affect plant cell turgor pressure.
  2. Test one of the four solutions from Procedure 2 and find out if what was predicted happens. Ask other students what they saw when done.
  3. Record all procedures, calculations, and observations.

Data (Results)

Procedure 1:

Potato 1

Potato 2

Potato 3

1) 0.5cm cell	2) 1cm cell	3) 1.5cm cell
Surface area: $1.5cm^2$	SA: $6cm^2$	SA: $13.5cm^2$
Volume: $0.125cm^3$	V: $1cm^3$	V: $3.375cm^3$
Ratio: 1.5 : 0.125	R: 6 : 1	R: 13.5 : 3.375

Procedure 2

Inside the cell	Outside the cell
15ml Glucose	100ml NaCl
15ml Albumin	100ml Sucrose
15ml Sucrose	100ml Glucose
15ml NaCl	100ml Albumin
15ml water	100ml Water

	Initial Cell Weight	Final weight
1M glucose solution :	27.9g →	28.6g
5% albumin solution :	26.7g →	28.4g
1M sucrose solution :	29.5g →	30.3g
1M NaCl solution :	27g →	28g
water :	26g →	27.7g

Percent Change in Weight

Glucose cell : +2.5% (+0.7g)

Albumin cell : +6.4% (+1.7g)

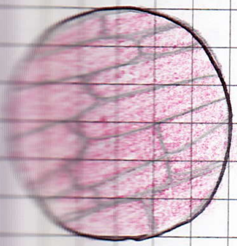
Sucrose cell : +2.7% (+0.8g)

NaCl cell : +3.7% (+1g)

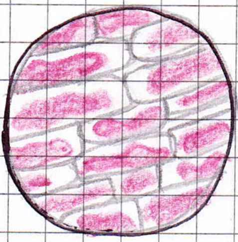
Water cell : +6.5% (+1.7g)

Procedure 3

Red onion



Red onion with glucose solution



Error Analysis

Mistakes may have been made while measuring the exact amount of solution to put into the cells in Procedure 2. The measurements had to be adjusted to correspond with the size of the plastic bag. Mistakes in weighing could have been made as well.

Conclusion

Part 1

The hypothesis was accurate, because the iodine diffused farthest into the smallest potato piece. This was observed after the three potato pieces had been submerged in iodine solution for almost 30 minutes. The largest potato had the biggest amount of untouched mass, compared to the other two potatoes. The iodine solution had diffused all the way to the middle of the smallest potato; in the medium potato, less so. The solution coated each potato and turned the outside into a black color and as time passed, seeped further into each "cell". The iodine represented the "food" that was entering the cell. The larger the cell became, the less nutrition it was getting in a certain amount of time. This is the reason that cells are so small, since the surface area to volume ratio is then much smaller, and therefore, nutrition (food) will get to the middle of the cell faster, which contributes to the efficiency of cells. As cell size increases, the surface area rapidly increases as well, making it harder for the food to get into the cell, because food would not be able to cross the membrane fast enough to support the increased volume. (The volume increases faster than surface area.)

Part 2

In the second part of the lab, students observed diffusion in model cells made of plastic bags. The hypothesis was that water would diffuse from the solution with higher free water potential to the solution with lower free water potential. This may have happened, since each cell experienced an increase in weight. However, this may also have been due to the diffusion of solute molecules from the corresponding solutions surrounding the cell, not just water.

The albumin cell in sucrose and the control (water in water) had the largest increase in weight. The slowest rate of diffusion was observed in the glucose cell in salt solution and the sucrose cell in glucose solution. The NaCl cell in albumin solution experienced an increase of one gram. It can be observed that each cell gained liquid from the surrounding solution, however it could not be determined exactly if only water, or only solute, or both diffused into the cell. It may have been easier to put each cell in the same solution, because a more accurate conclusion could be made about the rate of diffusion in or out of the cell, and the direction of diffusion. If students could have tested the molarity of the solutions after leaving the cells to diffuse for 30 minutes, the direction of diffusion ~~could~~ could probably also have been determined. Also, the permeability of the plastic bags most likely played a role in the rate of diffusion as well. Results could have been different with the use of different bags.

see continuation on next page

Part 3

In Part 3 students used slides and a light microscope to observe the effects of glucose solution on red onion cells. The hypothesis that water would enter or leave the cell due to solute concentration on the outside of the cell was correct. The assumption that turgor pressure would increase if the solute concentration on the inside of the cells was higher was also correct, except that the opposite happened, because solute concentration was higher on the outside after glucose solution was added.

The untouched onion cells were a purple color that was uniform throughout the cells. The cell membrane was very close to the cell wall, because the two structures were not very easy to differentiate. After ~~the~~ a few drops of glucose solution were added to the thin layer of onion on the slide and observed again under the microscope, a change had taken place. The purple color had "retreated" towards the inside of the cells. The cell membrane shrank away from the cell wall and the outline was clearly visible. It can be concluded that the turgor pressure decreased, because water left the cell through osmosis, since the concentration of solute (glucose) was much higher outside of the cells. This means that the solution inside the cell had higher free water concentration and therefore diffused into the surrounding solution. Students could clearly see how the cell had plasmolyzed. The glucose solution was hypertonic to the red onion cells.