Investigation #4: Diffusion & Osmosis

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Teacher’s Notes

Lab procedure adapted from College Board AP Biology Investigative Labs: An Inquiry Approach Teacher’s Manual

Click here for CB AP Biology Teacher Manual

Investigation #4
Diffusion and Osmosis

www.njctl.org
Pre-Lab

What causes my plants to wilt if I forget to water them?

In this lab we will:
· Investigate the relationship among surface area, volume, and the rate of diffusion.
· Design experiments to measure the rate of osmosis in a model system.
· Investigate osmosis in plant cells.
· Design an experiment to measure water potential in plant cells.
· Analyze the data collected in the experiments and make predictions about molecular movement through cellular membranes.
· Work collaboratively to design experiments and analyze results.
· Connect the concepts of diffusion and osmosis to the cell structure and function.

Pre-Lab Questions
Read the background information and answer the following questions in your lab notebook. (from pages S54-S55 of student lab manual)

1. Calculate the solute potential of a 0.1 M NaCl solution at 25°C. If the concentration of NaCl inside the plant is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M solution?
2. What must the turgor pressure equal if there is no net diffusion between the solution and the cell?
3. What is kinetic energy, and how does it differ from potential energy?
4. What environmental factors affect kinetic energy and diffusion?
5. How do these factors alter diffusion rates?
6. Why are gradients important in diffusion and osmosis?
7. What is the explanation for the fact that most cells are small and have cell membranes with many convolutions?
8. Will water move into or out of a plant cell if the cell has a higher water potential than the surrounding environment?
9. What would happen if you applied saltwater to a plant?
10. How does a plant cell control its internal (turgor) pressure?

Guided Investigation - Procedure 1

Materials - Procedure 1
· 2% agar containing NaOH and the pH-indicator dye phenolphthalein
· 1% phenolphthalein solution
· 0.1 M HCl or vinegar
· 0.1 M NaOH
· Cutting tools, such as squares of hard, thin plastic; unserrated knives; or scalpels
· Metric rulers
· Petri dishes
· Test tubes
· Lab notebooks

Safety
You must wear safety glasses or goggles, aprons, and gloves because you will be working with acids and caustic chemicals.

The HCl and NaOH solution will cause chemical burns, and you should use these solutions in spill-proof trays or pans.

Follow your teacher’s instruction carefully.

Do not work in the laboratory without your teacher’s supervision.

Talk to your teacher if you have any questions or concerns about the experiments.
Procedure 1: Surface Area and Cell Size

Cell size and shape are important factors in determining the rate of diffusion. Think about cells with specialized functions, such as the epithelial cells that line the small intestine or plant root hairs.

- What is the shape of these cells?
- What size are these cells?
- How do small intestinal epithelial and root hair cells function in nutrient procurement?

Procedure 1: Surface Area and Cell Size

Step 1 Place some phenolphthalein in two test tubes. Add 0.1 M HCl or vinegar to one test tube, swirl to mix the solutions, and observe the color.

Using the same procedure, add 0.1 M NaOH to the other test tube. Remember to record your observations.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td></td>
</tr>
</tbody>
</table>

Procedure 1: Surface Area and Cell Size

Step 2 Using a dull knife or a thin strip of hard plastic, cut three blocks of agar of different sizes.

These three blocks will be your models for your cells.

<table>
<thead>
<tr>
<th>Block</th>
<th>Surface Area</th>
<th>Volume</th>
<th>SA:V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block the fastest? Slowest? How do you explain the difference?

Analyzing & Evaluating Results: Procedure 1

Analysis Questions:
- Why are most cells small, and why do they have cell membranes with many convolutions?
- What organelles inside the cell have membranes with many convolutions? Why?
- If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block the fastest? Slowest? How do you explain the difference?

Designing & Conducting Your Investigation

Using the material listed earlier, design an experiment to test the predictions you made in Step 2.

Once you have finished planning your experiment, have your teacher check your design. When you have an approved design, run your experiment and record your results.

Do your experimental results support your predictions?
Guided Investigation - Procedure 2

Materials - Procedure 2

- Distilled or tap water
- 1 M sucrose
- 1 M NaCl
- 1 M glucose
- 5% ovalbumin (egg white protein)
- Cups
- Balances
- Lab notebooks

Procedure 2: Modeling Diffusion & Osmosis

You are in the hospital and need intravenous fluids. You read the label on the IV bag which lists all of the solutes in the water.

- Why is it important for an IV to have salts in it?
- What would happen if you were given pure water in an IV?
- How would you determine the best concentration of solutes to give a patient in need of fluids before you introduced the fluids into the patient’s body?

In this experiment, you will create models of living cells using dialysis tubing. Like cell membranes, dialysis tubing is made from a material that is selectively permeable to water and some solutes. You will fill your model cell with different solutions and determine the rate of diffusion.

Step 1
Choose up to four pairs of different solution. One solution from each pair will be in the model cell of dialysis tubing and the other will be outside the cell in the cup. Your fifth model will have water inside and outside; this is your control.

Before starting, use your knowledge about solute gradients to predict whether the water will diffuse into or out of the cell. Make sure you label the cups to indicate what solution is inside the cell and inside the cup.

<table>
<thead>
<tr>
<th>Inside</th>
<th>Outside</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

Step 2
Make dialysis tubing cells by tying a knot in one end of five pieces of dialysis tubing. Fill each “cell” with 10 mL of the solution you chose for the inside, and knot the other end, leaving enough space for water to diffuse into the cell.

Step 3
Weigh each cell, record the initial weight, and then place it into a cup filled with the second solution for that pair. Weigh each cell after 30 minutes and record the final weight.

<table>
<thead>
<tr>
<th>Pair (Inside/Outside)</th>
<th>Initial Weight</th>
<th>Final Weight</th>
</tr>
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<td></td>
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</tbody>
</table>

Step 4
Calculate the percent change in weight using the following formula:

\[(\text{final} - \text{initial})/\text{initial} \times 100\]

Record your results.
Analyzing & Evaluating Results: Procedure 2

Analysis Questions

- Which pair(s) that you tested did not have a change in weight? How can you explain this?
- If you compared 1 M solutions, was a 1 M NaCl solution more or less hypertonic than a 1 M sucrose solution? What is your evidence? What about 1 M NaCl and 1 M glucose and 1 M sucrose?
- Does the protein solution have a high molarity? What is the evidence for your conclusion?
- How could you test for diffusion of glucose?
- Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?

Independent Inquiry - Procedure 2

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Designing & Conducting Your Investigation

Using the available materials, design an investigation to answer one of the questions that came to your mind during the guided practice. Have your teacher check your design first. Remember to record your results, and be sure to use appropriate controls.

These questions can help jump-start your thinking.

- What factors determine the rate and direction of osmosis?
- What would you predict if you used a starch solution instead of protein?
- Can you diagram the flow of water based upon the contents of your model cell and the surrounding solution?
- When will the net osmosis rate equal zero in your model cells? Will it ever truly be zero?
- Based upon your observations, can you predict the direction of osmosis in living cell when the cells are placed in various solutions?
- How is the dialysis tubing functionally different from a cellular membrane?

Guided Investigation - Procedure 3

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Materials - Procedure 3

- Elodea (a water plant) or Mnium hornum (a moss)
- Light microscope
- Slides
- Dropper
- 1 M sucrose
- 1 M NaCl
- 1 M glucose
- 5% ovalbumin
- Lab notebooks

Procedure 3: Observing Osmosis in Living Cells

The interactions between selectively permeable membranes, water, and solutes are important in cellular and organismal functions. For example, water and nutrients move from plant roots to the leaves and shoots because of difference in water potentials. Based up what you know and what you have learned about osmosis, diffusion, and water potential in the course of your investigations, think about these questions.

- What would happen if you applied saltwater to the roots of a plant? Why?
- What are two different ways a plant could control turgor pressure, a name from internal water potential within its cells? Is this a sufficient definition for turgor pressure?
- Will water move into or out of a plant cell if the cell has a higher water potential than its surrounding environment?
### Procedure 3: Observing Osmosis in Living Cells

**Step 1** Start by looking at a single leaf blade from either *Elodea* or a leaf-like structure from *Mnium hornum* under the light microscope.
- Where is the cell membrane in relation to the cell wall? Can you see the two structures easily? Why or why not?
- What parts of the cell that you see control the water concentration inside the cell?

Back in Procedure 2 you tested diffusion and osmosis properties of several solutions. Now you are going to determine how they affect plant cell turgor pressure.
- What changes do you expect to see when the cells are exposed to the solutions?
- How will you know if a particular treatment is increasing turgor pressure? If it is reducing turgor pressure?
- How could you determine which solution is isotonic to the cells?

### Procedure 3: Observing Osmosis in Living Cells

**Step 2** Test one of the four solutions from Procedure 1 and find out if what you predicted is what happens. When you are done, ask other students what they saw. Be sure to record all of your observations.

### Independent Inquiry

**Materials - Independent Inquiry**
- Potatoes, sweet potatoes, or yams
- Cork borers or french fry cutter
- Balances
- Metric rulers
- Cups
- Color-coded sucrose solutions of different, but unlabeled, concentrations
- Lab notebooks

### Designing & Conducting Your Investigation

Design an experiment to identify the concentrations of the sucrose solutions and use the solution to determine the water potential of the plant tissues.

Use the following questions to guide your investigation.
- How can you measure the plant pieces to determine the rate of osmosis?
- How would you calculate the water potential in the cells?
- Which solution had a water potential equal to that of the plant cells? How do you know?
- Was the water potential in the different plants the same?
- How does this compare to your previous determinations in the *Elodea* cells?
- What would your results be if the potato were placed in a dry area for several days before your experiment?
- When the potatoes are in the ground, do they swell with water when it rains? If not, how do you explain that, and if so, what would be the advantage or disadvantage?