

## Lab 4. Diffusion and Osmosis in Selectively Permeable Membranes

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### Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures for each part of the experiment, and then answer the prelab questions at the end of this lab handout. Hand in the prelab assignment just *before* the start of your scheduled lab period.

### Goals of this Lab

After completing this lab exercise you should be able to.....

- Distinguish between *diffusion* and *osmosis*.
- Describe the mechanism of diffusion and osmosis at the molecular level.
- Explain why diffusion and osmosis are important to cells.
- Describe what is meant by *selectively permeable* and explain the role of a selectively permeable membrane in osmosis.
- Determine the effects of concentration and temperature on diffusion and osmosis.
- Describe the effects of hypertonic, isotonic, and hypotonic solutions on animal and plant cells.
- Explain how the presence of a cell wall affects osmotic behavior of plant cells.

### Introduction

All living things must obtain certain materials from their environment. For example, most animal cells obtain nutrients, oxygen, and water from their surroundings. In addition, they must get rid of waste materials. This exchange of substances between the cell and its environment is critical to survival and is dependent upon these materials being dissolved in water. Water is a great environment for life. Without it, life as we know it is impossible. If, as suspected, life in our solar system exists only on earth, it is probably because ours is the only planet known to possess liquid water on its surface.

The cytoplasm and extracellular environment of living cells are aqueous. The cytoplasm of living cells is composed of water (~70% to 80% water), which is the ***solvent*** or dissolving agent, and numerous dissolved ***solutes*** (e.g. salts, sugars, amino acids, vitamins, etc.). Virtually all substances entering and leaving cells are dissolved in water. The combination of a solvent and dissolved solute(s) is a ***solution***.

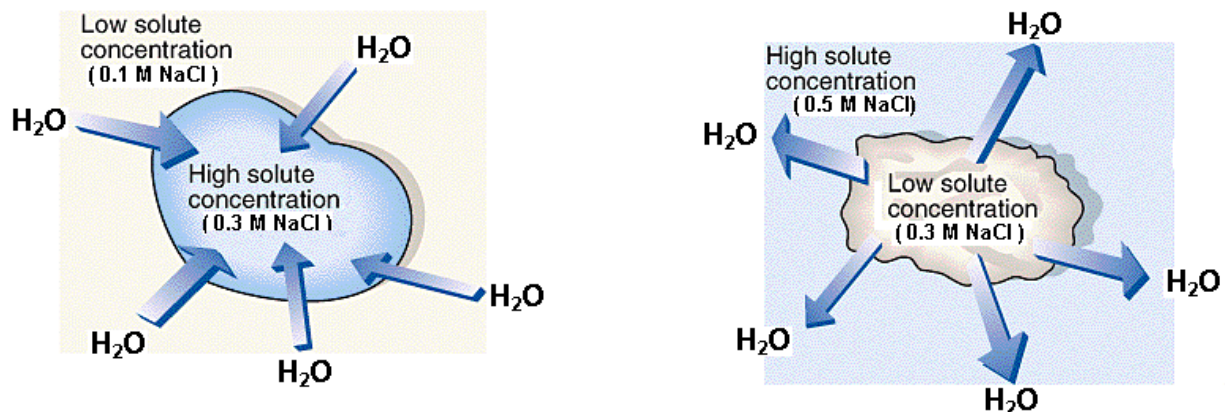
To perform their functions, cells must maintain a steady state in the midst of an ever-changing environment. This constancy is maintained by the regulation of the movement of solutes into and out of the cell. To achieve this control, a delicate membrane composed of a phospholipid bilayer that contains embedded and surface proteins bound cells. ***Cell membranes*** (also called plasma membranes) can distinguish different substances, slowing or inhibiting the movement of some while allowing others to pass through. Because not all substances penetrate the membrane equally well, cell membranes are said to be ***selectively permeable*** (or differentially permeable), allowing some substances to pass through easily, while completely or partially excluding others.

Although there are several methods by which solutes may enter or leave cells, the most common is diffusion. ***Diffusion*** is the movement of a substance (e.g. molecules and ions) from a region of higher concentration to one of lower concentration. Diffusion is a ***passive process***, meaning that it occurs without the expenditure of energy. The energy driving diffusion comes only from the intrinsic ***kinetic energy*** found in all atoms, ions, and molecules. If nothing obstructs the movement, a solute will diffuse until it spreads itself out uniformly and reaches a dynamic equilibrium.

So far, we've only described the movement of solutes across membranes. However, water (the solvent) also moves across cell membranes. The movement of *water* across a selectively permeable membrane from a region where *water* is highly concentrated to a region where its concentration is lower is known as **osmosis**. Osmosis is a special kind of diffusion, the diffusion of water.

In osmosis water *always* moves by diffusion across a selectively permeable membrane from a hypotonic solution (low solute concentration, high water conc.) to a hypertonic solution (high solute concentration, low water conc.). A **hypertonic solution** has a high solute concentration (and therefore a low concentration of water) relative to another solution. This is because when a solute is dissolved in water, its molecules (or ions) fill the space previously occupied by water molecules, thus reducing the concentration of water. On the other hand, a **hypotonic solution** has a low solute concentration (and thus a high concentration of water) relative to another solution because in the absence of large amounts of solute there is more space available for water molecules. Hence, osmosis can also be defined as the diffusion of water molecules across a selectively permeable membrane from a dilute solution (low solute conc. = hypotonic) to one that is concentrated (high solute conc. = hypertonic).

A cell placed into a hypertonic solution will shrink due to the loss of water. If too much water is lost, cell death may result. On the other hand, if a cell is placed in a hypotonic solution, water will enter the cell, causing it to swell and possibly burst. A cell placed in an **isotonic solution**, one that has the same solute concentration as another solution, the cytoplasm in this case, will not gain or lose water because water molecules will enter and leave the cell at equal rates.



- A. More water enters the cell than leaves the cell: There is a *net* movement of water from the *hypotonic* extracellular environment (lower solute conc., higher water conc.) to the *hypertonic* cytoplasm (high solute conc., low water conc.).
- B. **Crenation:** More water leaves the cell than enters the cell: There is a *net* movement of water from the *hypotonic* cytoplasm to the *hypertonic* extracellular environment

**Figure 1.** The two diagrams illustrate the process of osmosis, the diffusion of water across a selectively permeable membrane from a hypotonic to a hypertonic solution. The membrane is *impermeable* to the solute in these examples.

The difference in concentration of like molecules in two regions (e.g. on each side of a cell membrane) is called a **concentration gradient**. Diffusion and osmosis take place *down* concentration gradients, that is, from an area of high concentration to an area of low concentration. Over time, the concentration of the solvent and solute particles becomes equally distributed, and the gradient ceases to exist. At this point the system is said to be at equilibrium. At equilibrium solute and solvent continue to move randomly in all directions, but with no net change in their concentration.

This lab investigation will introduce you to the principles of diffusion and osmosis and study their underlying causes and some of the factors regulating these processes.

## PROCEDURE

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**Important Note!!** To get an overview of this laboratory activity and to use your lab time efficiently read the procedures for parts A-E *before* attending lab. If you and your group members are not familiar with these procedures before coming to lab you will have great difficulty completing this exercise during the lab period.

### Part A. Diffusion and Osmosis through a Selectively Permeable Membrane

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#### Introduction

Working in teams of four students, you will study the selective permeability of dialysis tubing. Dialysis tubing is a membrane made from cellulose that has numerous tiny invisible pores of a specific size. The permeability of dialysis tubing to the following substances will be investigated: water (18 g/mol), glucose (180 g/mol), starch (polymer of glucose), and I<sub>2</sub>KI. Iodine potassium iodide dissociates in aqueous solution into molecules of iodine, I<sub>2</sub>, (254 g/mol), and the following ions: K<sup>+</sup> (39 g/mol) and I<sup>-</sup> (127 g/mol).

The following chemical tests will be used in this part of the experiment:

- **I<sub>2</sub>KI Test for Starch:** When I<sub>2</sub>KI is added to an unknown solution, the solution turns a purplish or black color if starch is present. If no starch is present, the color remains a pale yellow-amber color, the color of dilute I<sub>2</sub>KI .
- **Benedict 's test for Reducing Sugars**<sup>1</sup>: When Benedict's reagent is added to an unknown solution and the solution is heated, the solution turns green, orange, or red if a reducing sugar such as glucose is present. This test is semi-quantitative. A green color indicates a relatively low concentration, while a red color results if the concentration of reducing sugar is relatively high. The color remains blue, the color of Benedict's reagent if no reducing sugars are present.

<sup>1</sup>For those budding biochemists and organic chemists amongst us, reducing sugars have an aldehyde group that is free to be oxidized to a carboxyl group. Most monosaccharides, e.g. glucose, are reducing sugars, most disaccharides, e.g. sucrose, are not.)

## MATERIALS

### Per Group of 4 Students:

2- 400 mL beakers  
25 cm length of moist dialysis tubing:  
(soaked in DI water)  
String  
Small funnel  
10 mL graduated cylinder  
Laboratory balance  
Boiling water bath  
3 - Standard size test tubes  
Safety goggles

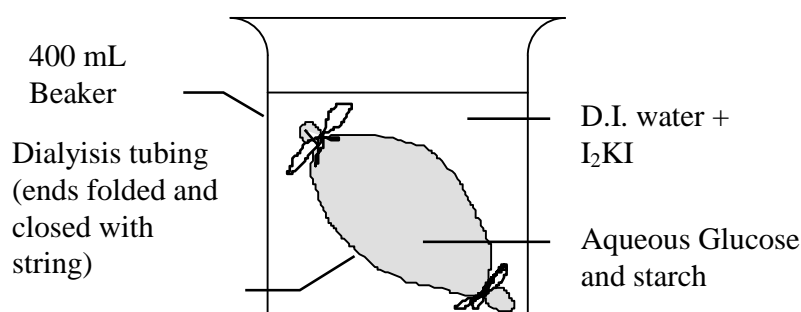
Test tube holder (hand held type)  
Test tube rack  
Eye Droppers  
Waste container for used Benedict's Reagent

### Solutions

40% Glucose solution  
Starch solution  
I<sub>2</sub>KI solution  
Benedict's reagent  
Vinegar for base spills

### Procedure (Work in Teams of Four)

1. As outlined below, construct the experimental set up illustrated in figure 2. Divide up the labor involved for the various steps amongst your group members.



**Figure 2.** Set-up for the investigation of the permeability of dialysis tubing. A 400 mL beaker will be used in this experiment.

### 2. Preparation of the Solution in the Dialysis Tubing

- a. Obtain a 25 cm length of dialysis tubing that has been soaked in deionized water, fold over about 3 cm of one end, and tie it securely with string to form a leak proof bag that is open at one end.
- b. Roll the open end of the tube between your thumb and forefinger until it opens, insert a funnel, and add about 10 mL of 40% Glucose solution, followed by about 10 mL of the starch solution.
- c. Close the open end of the bag: Press out the air, fold over the open end and tie it securely with string to form a closed leak proof sac.
- d. Rinse and dry the closed sac: Place the closed sac under running tap water to remove any solution from the outside. Carefully squeeze out excess water from the string at each end, and then blot the sac dry by rolling it on a paper towel.
- e. Mix the contents of the sac and record the color of its contents in the data table on the report sheet.
- f. Determine the mass of the sac in grams with a laboratory balance and record the result on the report sheet.

3. **Preparation of the I<sub>2</sub>KI Solution in the Beaker**

- Add about 200-250 mL of deionized water to a clean 400-mL beaker.
- While mixing, add several droppersfull of I<sub>2</sub>KI solution to the water until the contents of the beaker has a distinct light yellow color.
- Record the color of the beaker's contents on the report sheet.

4. **Place sac in the beaker**

- Place the sac prepared in step 1 in the beaker prepared in step 3. Be sure that the sac is completely covered by the I<sub>2</sub>KI solution in the beaker. If it is not entirely covered, then add more solution.
- Allow the sac to remain undisturbed in the beaker for 45-60 minutes.** After this time the sac will be dried and reweighed. The sac's contents and the surrounding solution will be observed for color changes, and then chemically analyzed for the presence of glucose.

5. **Preparation of Boiling Water Bath**

- Prepare a boiling water bath that will be ready for use after the sac has soaked for 45-60 minutes: Place a couple of boiling chips in a 400 mL Beaker, fill half way with tap water, and place on a hot plate turned to its highest setting. Once the water comes to a boil, reduce the heat so the water simmers gently.

|   |
|---|
| <p><b>Tip!!</b>                      <b>While the dialysis sac soaks for 45-60 minutes</b><br/><b>Proceed to Parts B, C, D, and E, then return to steps 6-8, below.</b></p> |
|---|

6. **Observations and Reweighing of the Sac**

- After 45 to 60 minutes, remove the dialysis sac, rinse and dry as in step 2, above: Rinse with tap water. Carefully squeeze out excess water from the string at each end, then blot the sac dry by rolling it on a paper towel.
- Weigh the sac and its contents and record the mass in grams on the report sheet.
- Record the final colors of the sac and the contents of the beaker on the report sheet.

7. **Benedict's Test for the Presence of Sugar in the Solutions**

|  |
|--|
| <p><b>Caution!!</b>                      Benedict's Reagent is very caustic. It can burn holes in clothing and digest skin!! <b>Wear goggles for eye protection!!</b>. Clean up spills immediately after first neutralizing with vinegar. If spilled on your skin (it feels slippery and begins to burn after several minutes) wash thoroughly with tap water. Report all accidents.</p> |
|--|

- Label three clean test tube: control, sac, and beaker. Add one dropperful of Benedict's reagent to each test tube.
- Use a clean eyedropper to put 2 droppersful of DI water in the "control" test tube.
- Use scissors to cut off one end of the sac. With a clean eye dropper, put 2 droppersful of Sac solution in the "sac" test tube.
- Use a clean eyedropper to put 2 droppersful of beaker solution in the "beaker" test tube .
- Heat the test tubes in a *boiling* water bath for about 3 minutes.
- Record the colors of the solutions on the report sheet.

8. **Clean up.....**

- a. Dispose of the used and excess Benedict's reagent in the "Benedict's Waste" container located on the lab cart.
- b. Wash down the drain with tap water all other solutions.
- c. Rinse all glassware with tap water before returning to the lab cart.
- d. Place used dialysis tubing and string in a wastebasket.

**Part B. Brownian movement of a Carmine Powder Suspension**

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**Introduction**

In this part of the lab exercise we will study *Brownian movement*, the random movement of large particles in a suspension brought upon by the transfer of kinetic energy when the much smaller solvent molecules collide with them. Brownian movement, the driving force responsible for diffusion, was first observed in 1827 by the Scottish botanist Robert Brown as he observed pollen grains suspended in water on a slide appear to move by some unexplained force. Albert Einstein, when searching for evidence to support the existence of atoms and molecules in 1905, predicted that this type of random motion must exist, although he was unaware that it had been observed many years before. It was only after the kinetic molecular theory was established years later that the mechanism of Brownian movement was finally understood.

Although we often think of living matter as having special properties, it is in fact, subject to the same physical principles as all other matter. The molecules of any liquid are in constant motion. Since the cytoplasm of a cell is approximately 70-80% water, the constant random motion of the water molecules within the cytoplasm helps to distribute substances within a cell.

**MATERIALS**

**Per Team of Two Students:**

- |                                |                   |
|--------------------------------|-------------------|
| Compound microscope            | Dissecting needle |
| Depression slide and coverslip | Carmine powder    |
| Eye dropper and water          |                   |

**Procedure** (Work in teams of two)

1. Work in Pairs: One person set up the microscope while the other makes the wet mount of carmine powder, outlined below.
2. **Make a Wet Mount of Carmine Powder**
  - a. Place a drop of water in the depression of a depression slide.
  - b. Barely place the tip of a dissecting needle in the drop and then into the dry carmine powder.
  - c. Dip the tip of the dissecting needle into the drop of water on the slide, mix, and cover with a coverslip.

|   |
|---|
| <p><b>Tip!!!</b> You should use such a tiny amount of carmine powder that a pink color is barely noticeable when it is mixed thoroughly in the drop of water.</p> |
|---|

3. Examine the slide under low power and then high power. Focus your attention on the movement of one or two very small particles and a couple of larger particles.
4. Record your observations on the report sheet. Consider the following when writing your describing *your* observations.....Is the movement random or only in one direction? Is the movement continuous or does it occasionally stop? How does the movement of large particles compare to the movement of small particles?
5. Clean the microscope slide and return it to the front table after discarding the coverslip. Keep the microscope for part D or the experiment.

### **Part C. Effect of Temperature and Solute Size on the Rate of Diffusion of Dyes through Gelatin**

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#### **Introduction**

Solutes move within a cell's cytoplasm largely because of diffusion. However, the rate of diffusion (the distance moved in a given amount of time) is affected by factors such as temperature and the size of solute particles. In this part of the lab exercise we will investigate how these two factors influence the rate of diffusion. Since the cytoplasm of a cell is a colloid<sup>1</sup>, a common colloid, gelatin (consists of the protein keratin, the stuff hair, nails, skin, and Jello™ are made of), will be used to simulate the cytoplasm of a cell.

<sup>1</sup> A colloid is a homogeneous mixture in which the particles are too large to be dissolved but too small to settle out as a solid precipitate. e.g. Muddy water is an example of a colloid where the dirt particles are too large to dissolve, but too small to settle to the bottom. Likewise, many cytoplasmic proteins are too large to dissolve, but too small to settle out of the mixture.

#### **MATERIALS**

Metric ruler (one per student)

##### **Per group of 4 students:**

One set of three screw top test tubes half-filled with 5% gelatin at 5 °C to which a crystal of the following dyes have been added:

- # 1: Potassium dichromate (5 °C)
- # 2: Janus green (5 °C)
- # 3: Aniline Blue (5 °C)

##### **Per group of 4 students:**

One set of three screw top test tubes as on the left, but at room temperature:

- # 4: Potassium dichromate (rm. temp.)
- #5: Janus green (room temp.)
- #6: Aniline Blue (room temp.)

#### **Procedure** (Work in teams of four)

1. Two sets of three screw-cap test tubes have been half-filled with 5% gelatin. One mL of dye has been added to each test tube.
  - Obtain one set from the refrigerator and another from the lab cart.
  - Your instructor has written on the front board the time at which the experiment was started. Record this time on the report sheet.

- Distribute the tubes to the group members: Now hold each tube vertically in front of a white sheet of paper.
  - Each person in the group should use a metric ruler to measure in millimeters the distance the dye has diffused from the gelatin's surface in each tube. Record the results on the report sheet.
  - Record on the report sheet the time (to the nearest minute) at which the distances were measured.
- Return set 1 (tubes 1-3) to the refrigerator, and set 2 (tubes 4-6) to the lab cart.

## Part D. Osmotic Behavior of Cells with a Cell Wall: Purple Onion Cells

### Introduction

**Tonicity** is a description of one solution's solute concentration compared to that of another solution. A **hypotonic solution** is a solution containing a **lower** concentration of **solute** particles than another solution. Solutions containing **equal** concentrations of **solute** are **isotonic** to each other, while a **hypertonic solution** is one containing a **greater** concentration of **solute** than another solution. Osmosis is the diffusion of water across a semipermeable membrane from a hypotonic solution to a hypertonic solution.

In their natural environment, the cells of freshwater plants and algae are surrounded by a hypotonic extracellular fluid, which results in a net flow of water into the cells. The presence of a cell wall, a rigid structure surrounding the plasma membrane that is freely permeable to water and most solutes, prevents plant and algal cells from bursting when surrounded by a hypotonic solution. There is build up of **turgor pressure**<sup>1</sup> as water moves into the cell and eventually into the hypertonic central vacuole where sugars produced by photosynthesis are stored. High turgor pressure prevents the further uptake of water by the cell (See figure 3A). On the other hand, if the turgor pressure within a plant's cells should drop due to the loss of water, the plant will wilt as a consequence.

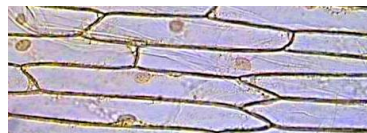
Plant cells, like all cells, when placed in a hypertonic environment will lose water, causing the cells to become dehydrated or **plasmolyzed**. In plants, this process is called **plasmolysis** (in animals it is known as **crenation**). Plasmolyzed cells are easily identified by the separation of the cell membrane and the enclosed cytoplasm from the cell wall due to the lack of turgor pressure (See figure 3B). In fully turgid cells the cell membrane is not visible as it is pressed tightly against the cell wall by the high turgor pressure of the cytoplasm.

In this part of the experiment, three wet mounts of purple onion<sup>2</sup> epidermis will be made using unknown solutions A, B, and C. One is hypotonic (distilled water), one is hypertonic (20% NaCl), and another is isotonic (0.9% NaCl). Each group of four students will work together to identify the three unknown solutions by observing these slides under a compound microscope.

<sup>1</sup> **Turgor Pressure**: the pressure resulting from the uptake of water as the plasma membrane and the enclosed cytoplasm press against the cell wall of a plant or algal cell.

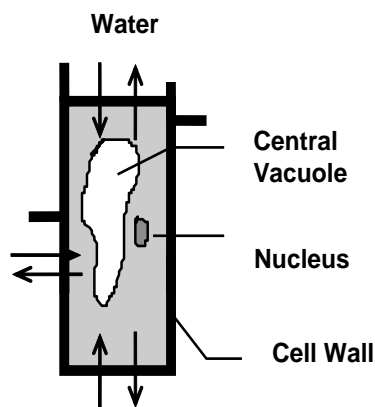
<sup>2</sup> Purple onions are ideal for this investigation because the plasma membrane of its cells encloses a purple colored cytoplasm. It is, therefore, easy to observe the separation of the plasma from the cell wall when the cells are placed in a hypertonic environment.

**Figure 3.** A photomicrograph of **purple onion epidermis**. Note the prominent **nucleus** in each cell. Is the surrounding solution hypertonic or hypotonic?





A. **A fully turgid plant cell in a hypotonic solution at Equilibrium.** Initially, more water enters the cell than leaves, causing an increase in turgor pressure that hinders the entry of water. Equilibrium will ultimately be established, a state in which water will enter and leave the cell at the same rate. The cell membrane is not visible as it is pressed firmly against the cell wall because of the high turgor pressure. The central vacuole stores sugars



B. **A plasmolyzed plant cell in a hypertonic solution.** The plasma membrane is clearly visible as it separates from the cell wall due to the low turgor pressure. Until equilibrium is established, more water leaves the cell than enters due to the hypertonic external environment.

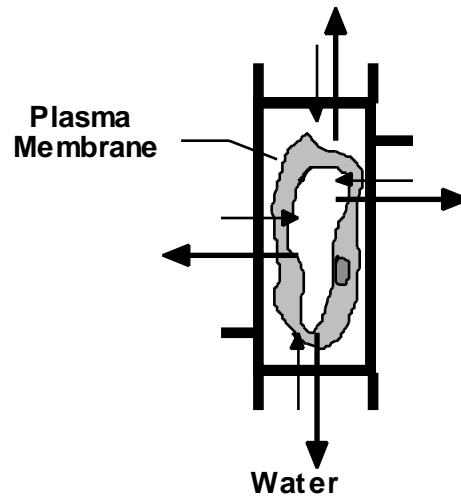


Figure 4. Turgid and plasmolyzed plant cells.

## MATERIALS

### Per Student:

Compound microscope  
One slide and coverslip  
Purple onion scale  
Forceps and scalpel

### Per Group of four Students

Unknown solutions in dropper bottles  
(labeled A, B, and C):  
Distilled water  
0.9% NaCl  
10% NaCl

**Procedure** (Work in teams of four)

1. Each group member should follow the procedure below to make a wet mount of the *colored* epidermis from a scale of a purple onion using one of the three unknown solutions A, B, or C.

|   |
|---|
| <b>Tip!!!</b> Work quickly to minimize dehydration of the epidermal tissue and changes in tonicity of the solutions due to evaporation. |
|---|

- a. Cut a purple onion bulb into quarters.
  - b. Remove one of the scales with a dark epidermal layer.
  - c. Snap the scale backward to produce a ragged piece of epidermis.
  - d. Use forceps to carefully remove a piece of purple epidermis and spread it evenly in a drop of solution A, B, or C. Try to avoid wrinkling the epidermis when spreading it on the slide.
  - e. Gently lower a coverslip to prevent trapping air bubbles.
  - f. Observe the preparation with low-, medium-, and high-power objectives. Record your observations on the report sheet.
2. Observe the slides prepared by the other members of your group and record your observations on the report sheet.
  3. Based on your microscopic observations of the onion epidermis, identify solutions A, B, and C in the discussion section of the report sheet.
  4. Clean the slides and return them to the front table
  5. Return your microscope to the cabinet after returning it to scanning power and winding up its cord.

**Part E. Osmotic Behavior of Animal Cells: Human Blood Cells  
(Teacher Demonstration)**

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**Introduction**

Animal cells lack the rigid cell wall of a plant. The external boundary of an animal cell is the differentially permeable plasma membrane. Consequently, an animal cell increases in size as water enters the cell. However, since the plasma membrane is relatively fragile, it ruptures when too much water enters the cell. This is because of excessive pressure pushing against the plasma membrane. Conversely, if water moves out of the cell, it will crenate and look spiny.

In this part of the experiment, three wet mounts of human blood (your instructor's!) will be made using the unknown solutions D, E, and F. One is hypotonic (distilled water), one is hypertonic (10% NaCl), and another is isotonic (0.9% NaCl). Your instructor will demonstrate this for you using a microscope connected to a video camera and a monitor.

**MATERIALS**

**Solutions:**

Unknown solutions in dropper bottles (labeled D, E, and F):  
Distilled water  
0.9% NaCl  
10% NaCl

**Equipment**

Compound microscope connected to video camera and monitor  
4 Slides and coverslips  
Blood lancet and alcohol wipes  
Autoclave bag

**Procedure (Teacher Demonstration)**

1. **Observation of Control Slide: Human Blood without Treatment.**

- a. Place a drop of blood on a slide and gently lower a coverslip to prevent trapping air bubbles.
- b. Observe the shape of the red blood cells with no treatment at low and high power. Record your observations on the report sheet.

2. **Observation of human blood in unknown solutions D, E, and F.**

- a. Put a drop of Solution D, E, or F on a labeled slide followed by a drop of blood and gently lower a coverslip to prevent trapping air bubbles.

|                    |  |
|--------------------|--|
| <b>Take Note!!</b> | If red blood cells swell and then burst, the ruptured skeleton of the plasma membrane will only be visible if viewed by phase contrast microscopy. |
|--------------------|--|

- b. Observe the shape of the red blood cells at low and high power. Record your observations on the report sheet.
3. Based on your microscopic observations of red blood cells, identify solutions D, E, and F in the discussion section of the report sheet.

*Everyone has photographic memory—some  
just don't have the film.*



*There is a fine line between fishing and just  
standing on the shore like an idiot.*

**Lab 4 Report Sheet**  
**Diffusion and Osmosis**  
**Biol 211**

Name \_\_\_\_\_  
 Group Number \_\_\_\_\_ Date \_\_\_\_\_

**Part A. Diffusion and Osmosis through a Selectively Permeable Membrane**

**Results**

**Table 1.** Data for the experiment investigating the permeability of dialysis tubing to water, starch, glucose, and I<sub>2</sub>KI.

| Source of Solution                         | Color                  |                       |                       | Mass of Sac (g) |               |
|--|------------------------|-----------------------|-----------------------|-----------------|---------------|
|  | Before Soaking the Sac | After Soaking the Sac | After Benedict's Test | Before Soaking  | After Soaking |
| Sac  |                        |                       |                       |                 |               |
| Beaker                                     |                        |                       |                       |                 |               |
| Control Tube<br>(Water and Benedicts Soln) |                        |                       |                       |                 |               |

**Discussion**

1. Explain why there was a change in mass of the sac. Use the terms diffusion and/or osmosis, hypotonic, isotonic, and/or hypertonic in your response.
  
2. Explain the behavior of the substances in this experiment by completing the table below.

| Substance         | Direction of Net Movement<br>(Into or out of sac?) | Reason for the net movement of substance |
|-------------------|--|--|
| I <sub>2</sub> KI |  |  |
| Glucose           |  |  |
| Starch            |  |  |
| Water             |  |  |





**Part C. Effect of Temperature and Solute Size on the Rate of Diffusion**

**Results**

**Table 2.** Times the dyes were allowed to diffuse in the gelatin.

|                         |  |
|-------------------------|--|
| Time experiment started |  |
| Time experiment ended   |  |
| Elapsed time in hours   |  |

**Table 3.** Results for the effect of temperature and solute size on the rate of diffusion.

| Solute (dye)         | MW <sup>1</sup> | Set 1 (5 °C)           |                           | Set 2 (room temperature) |                           |
|----------------------|-----------------|------------------------|---------------------------|--------------------------|---------------------------|
|                      |                 | Distance Diffused (mm) | Rate of Diffusion (mm/hr) | Distance Diffused (mm)   | Rate of Diffusion (mm/hr) |
| Potassium Dichromate | 294             |                        |                           |                          |                           |
| Janus Green          | 511             |                        |                           |                          |                           |
| Aniline Blue         | 738             |                        |                           |                          |                           |

<sup>1</sup>MW = Molecular weight, a reflection of the mass of the molecules or formula units of a substance. Measured in atomic mass units (a.m.u.) or in grams per mole.

**Discussion**

1. Use a calculator to determine the rate of diffusion in millimeters per hour for each dye at 5 °C and at room temperature. Record your rates in table 3, above, rounding to the correct number of significant figures. (Don't forget those good old sig. figs. from chemistry!!) You need *not* show your calculations.
2. Analyze the results as fully and completely as you can concerning the effect temperature and solute size has on the rate of diffusion. Use the experimental data to substantiate your analysis and any conclusions that you draw.



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**Part D. Osmotic Behavior of Cells with a Cell Wall: Purple Onion Cells**

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**Results**

**Table 3.** Appearance of purple onion epidermal cells in unknown solutions A, B, and C.

| <b>Unknown Soln.</b> | <b>Identity of Solution</b> | <b>Appearance/Condition of Cells</b> |
|----------------------|-----------------------------|--------------------------------------|
| <b>A</b>             |                             |                                      |
| <b>B</b>             |                             |                                      |
| <b>C</b>             |                             |                                      |

**Discussion**

1. Based on your observations of the three slides, which solution, A, B, or C.....
  - a. is Isotonic?
  
  
  
  
  
  
  
  
  
  
  - b. is Hypertonic?
  
  
  
  
  
  
  
  
  
  
  - c. is Hypotonic?
  
  
  
  
  
  
  
  
  
  
  - d. produced the greatest turgor pressure in the onion cells?
  
  
  
  
  
  
  
  
  
  
  - e. produced the least turgor pressure in the onion cells?

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**Part E. Osmotic Behavior of Cells Animal Cells: Red Blood Cells**

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**Results**

**Table 4.** Appearance of red blood cells: Untreated and in unknown Solutions D, E, and F.

| <b>Treatment</b>           | <b>Identity of Solution</b> | <b>Appearance/Condition of Cells</b> |
|----------------------------|-----------------------------|--------------------------------------|
| <b>Control (Untreated)</b> |                             |                                      |
| <b>Unknown D</b>           |                             |                                      |
| <b>Unknown E</b>           |                             |                                      |
| <b>Unknown F</b>           |                             |                                      |

**Discussion**

1. Based on your observations of the three slides, which solution, D, E, or F is.....
  - a. isotonic?
  
  
  
  
  
  
  
  
  
  
  - b. hypertonic?
  
  
  
  
  
  
  
  
  
  
  - c. hypotonic?
  
2. List factors that might cause the results to be different than what is expected?
  - a.
  
  
  
  
  
  
  
  
  
  
  - b.
  
  
  
  
  
  
  
  
  
  
  - c.



*Always remember you're unique, just like everyone else.*



*If you die in an elevator, be sure to push the Up button.*

**Lab 4: Prelab Questions**  
**Diffusion and Osmosis**  
**Biol 211**

Name \_\_\_\_\_  
Group Number \_\_\_\_\_ Date \_\_\_\_\_

**Note:** Do the prelab reading at the beginning of this lab handout *before* attempting to answer the questions that follow! Hand in this assignment just *before* the start of your scheduled lab period.

1. Develop a hypothesis for each part of the experiment by completing each section below. Note: Recall that a hypothesis is an *educated* guess that predicts the outcome of an experiment. Scientists often like to propose correct hypotheses (due to vanity??), but this, of course, is not always possible. More important than being correct, a hypothesis *must* be testable!

**Part A. Diffusion and Osmosis through a Selectively Permeable Membrane**

Select from the choices, A-D, to predict the behavior of I<sub>2</sub>KI, glucose, starch, and water.

|                         |                              |
|-------------------------|------------------------------|
| _____ I <sub>2</sub> KI | A. Move into the sac         |
| _____ Glucose           | B. Move out of the sac       |
| _____ Starch            | C. Remain outside of the sac |
| _____ Water             | D. Remain in the sac         |

**Part B. Brownian movement of a Carmine Powder Suspension**

Predict the relative behavior of the small and large carmine particles as you view them under the microscope:

**Part C. Effect of Temperature and Solute Size on the Rate of Diffusion of Dyes through Gelatin**

Predict the relative rates of diffusion for potassium dichromate, Janus green, and aniline blue at room temperature and 5 °C.

**Part D. Osmotic Behavior of Cells with a Cell Wall: Purple Onion Cells**

Predict the appearance/behavior of the purple onion epidermal cells when placed in.....

- Distilled water:
  
- 0.9% NaCl:
  
- 20% NaCl:

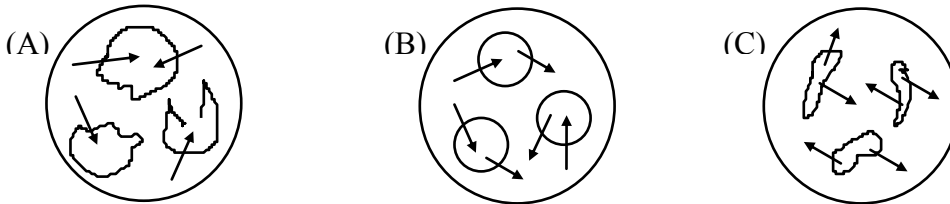
**Part E. Osmotic Behavior of Animal Cells: Red Blood Cells**

Predict the appearance/behavior of red blood cells when placed in.....

- Distilled water:
  
- 0.9% NaCl:
  
- 10% NaCl:

2. The diagrams below represent three microscopic fields containing cells of a freshwater protist. Arrows show the direction of net osmosis. Identify which field contains each solution indicated below. Use the letters of the fields of view as your choices and place them in the blanks.

- a. Which field contains a hypertonic extracellular solution? \_\_\_\_\_
  
- b. Which field contains an isotonic extracellular solution? \_\_\_\_\_
  
- c. Which field contains a hypotonic extracellular solution? \_\_\_\_\_



3. A sac made from dialysis tubing containing 4% NaCl, 9% Glucose, and 10% albumin (a protein found in blood plasma) is suspended in a beaker containing a solution with the following composition: 10% NaCl, 10% glucose, and 40% albumin. Assume that the sac is permeable to all substances except the albumin.

Select from the choices, A-D, to predict the behavior of NaCl, glucose, albumin, and water.

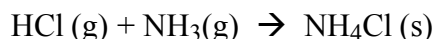
- |               |                              |
|---------------|------------------------------|
| _____ NaCl    | A. Move into the sac         |
| _____ Glucose | B. Move out of the sac       |
| _____ Albumin | C. Remain outside of the sac |
| _____ Water   | D. Remain in the sac         |

4. Suppose you would like to serve crisp lettuce, but your lettuce has become limp. To make it crisp again, in which of the following should you soak the lettuce? Circle the letter of the correct response and explain the reasoning behind your choice.

- A. Hypertonic solution (e.g. Salt water made from ~3 tablespoons of salt in a quart or water.
- B. Hypotonic solution (e.g. Distilled water)
- C. Isotonic solution (A solution with the same solute concentration as lettuce cells)

**Explanation:**

5. Use the following information to answer the questions "a" and "b" below. Assume you are conducting the experiment illustrated in figure 1, below. Both hydrochloric acid (hydrogen chloride gas, HCl, dissolved in water) and ammonia solution, (ammonia gas, NH<sub>3</sub>, dissolved in water) consist of a volatile solute that can easily leave the solution and enter the gaseous state. When gaseous molecules of hydrogen chloride and ammonia collide they react according to the reaction below to form Ammonium chloride, NH<sub>4</sub>Cl, a white solid. A smoky white solid deposited on the glass tubing will provide evidence for the reaction.



**Figure 1.** Sealed glass tube containing cotton balls that have been saturated with ammonia solution or hydrochloric acid, respectively.

Predict which gas will diffuse more quickly and indicate to which end of the tube the smoky precipitate will be closer.

**Hints:** Molecular weights: NH<sub>3</sub> = 17 amu, HCl = 36.5 amu; Molecules at the same temperature have the same average kinetic energy; K.E. =  $1/2mv^2$ ; i.e. for two different molecules, 1 and 2, at the same temperature:

$$1/2m_1v_1^2 = 1/2m_2v_2^2$$

- a. The faster diffusing gas is: HCl or NH<sub>3</sub> (circle one)

**Explain your reasoning:**

- b. At which end of the tube will the precipitate form closest to? HCl or NH<sub>3</sub> (circle one)

**Explain your reasoning:**