WARNING — This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.

Visualizing Osmosis and Diffusion

INVESTIGATION

#20–2253

Objectives

- ***Construct*** **models of cells, simulating the function of cell membranes with dialysis tubing**
- ***Observe*** **the selective permeability of cell membranes**
- ***Simulate*** **the process of osmosis and diffusion using a model cell**
- ***Predict*** **the characteristics of substances which allow them to pass through a semipermeable membrane**

Science Concepts

- Osmosis
- Diffusion
- Cell Membrane
- Solute
- Solvent
- Osmoregulation
- Plasmolysis

Kit Materials List

30	mL	Red dye solution
30	mL	Blue dye solution
20		Cups, clear
4	m	Dialysis tubing
250	mL	Glucose solution
50		Glucose test strips
60		Medicine cups
20		Pipets, plastic
30	mL	Starch indicator solution (IKI)
250	mL	Starch solution
4	m	String

Time Requirements

Demonstration
20 minutes

Lab Activity
45 minutes

Safety

Have your students always wear safety goggles, gloves and a lab apron to protect their eyes and clothing when working with any chemicals. Be sure that students wash their hands before leaving the laboratory.

Iodine Potassium Iodide (IKI) is a corrosive substance and can be a caustic irritant if allowed to come in contact with the skin. Keep the iodine potassium iodide bottle tightly capped and only open it when the students are ready to use it. In case of spills or skin contact, flush exposed areas with running water for 15 minutes.

Disposal of iodine potassium iodide (IKI) should be completed using the instructions on the MSDS.

Any waste starch and glucose solutions from this exercise may be disposed of by pouring them down the drain. Used dialysis tubing may be disposed of in a regular solid waste container.

Pre-Lab Preparation

Enough materials are provided in this kit for up to 40 students working in groups of two.

Cut the dialysis tubing provided into 15 cm segments and the string into 10 cm pieces. Place the dialysis segments into a cup of water for 2 minutes or until they are pliable.

Dispense 10 mL each of the starch and glucose solutions into the medicine cups provided in the kit to distribute them to each student team. Be sure to shake the starch solution before dispensing it to the students to ensure the proper suspension of the starch molecules in solution. Alternatively, you may want to have your students dispense the amounts needed using the medicine cups as a measuring device.

To reduce the possibility of iodine potassium spills, you may want to set up a station for students to dispense it directly into their test cup.

Lab Activity Procedures & Notes

Teacher demonstration

1

Demonstrating the Characteristics of Cell Membranes

What you need

30	mL	Red dye solution
30	mL	Blue dye solution
1		Cup, clear
6	in.	Dialysis tubing
		Plastic pipet
		String
		Water
		Graduated cylinder

What to do. . .

Step 1

Tie one end of a tubing tightly with string or tie a knot in one end. This end of the tubing should be tied tightly enough to prevent any leaks from the end of the bag.

Step 2

Fill the dialysis tubing with 10 mL each of the red and blue dye solutions and tie the open end tightly. The dialysis tubing simulates a cell membrane surrounding a cell.

Step 3

Place the filled dialysis tubing in a cup with 100 mL of water and allow it to stand for 15-20 minutes. Ask your students to predict what will happen to the contents inside the tubing and inside the cup.

Step 4

Carefully observe what is happening inside the cup. Record your observations in Data Table 1.

Step 5

At the end of 15-20 minutes, or longer if time allows, determine the volume of water remaining in the cup using a graduated cylinder. Have your students determine the final volume of water inside the dialysis tubing and record the results in Table 1.

Data Table #1

Observations	Solution in Cup	Solution in Tubing
Initial Color	**Clear**	**Red/Blue**
Final Color	**Light Blue**	**Red**
Initial Volume	**100 mL**	**10 mL**
Final Volume	**98 mL**	**12 mL**

Questions

1. Based upon your observations, what conclusions can you draw?

   The molecules of the blue dye are small enough to pass through the dialysis tubing, while the red dye molecules are too big to pass.

2. Explain any change in volume both in the cup and in the tubing after the dialysis process.

   Because the concentration of water is greater outside the tubing, water moves across the membrane down its concentration gradient and into the tubing.

3. How is this demonstration similar to the action of cell membranes?

   The dialysis tubing, like a cell membrane, is selectively permeable since it lets some substances into and out of the cell while not allowing other substances to pass at all.

4. Why didn't the red dye enter the cup with the water?

   The red dye molecule was too large to pass through the pores of the dialysis tubing.

Lab Activity
Procedures & Notes

Activity 2

Osmosis and Diffusion in Model Cells

What you need

Per Group

1		Cup, clear
15	cm	Dialysis tubing
10	mL	Glucose solution
1		Pipet, plastic
30	mL	Starch indicator solution (IKI)
10	mL	Starch solution
20	cm	String
		Water

Per Student

Apron
Gloves
Goggles

What to do. . .

Step 1

Have your students wear their apron, protective gloves and eye goggles.

Step 2

Your students shouhd then dip a glucose test strip into the water in the cup for 1-2 seconds. They should then run the test strip along the edge of the cup to remove any excess liquid. The students should wait approximately 2-3 minutes and observe any color change on the strip. A positive (+) glucose test is indicated by a greenish color to the test strip. No color change will occur if the test result is negative (-). They should record the results of the test in Data Table #1.

Step 3

Caution: *IKI solution is a poison. Avoid any skin contact. Be sure to wear proper safety equipment.*

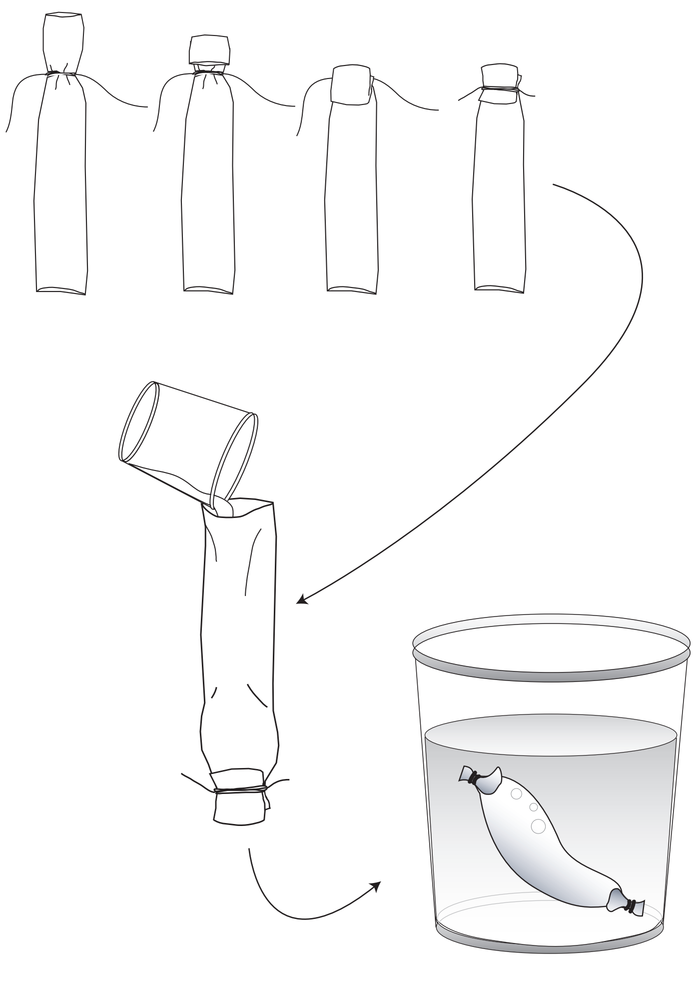
Students should carefully add 20-25 drops of the starch indicator solution (IKI) to the water in the cup. They should observe what happens to the indicator solution as it mixes with the water. They should record the color of the water in Data Table #1.

Step 4

Provide your students with a soaking dialysis tubing segment. They should gently rub the tubing between their fingers to open it.

Step 5

Your students should tie one end of the tubing tightly with string or tie a knot in one end. They fold the tubing over the string twice and then tie the string around the fold as illustrated in the figure below. They should make sure this end of the cell is tied tightly enough to prevent leaks from the end of the bag. They should then fill the tubing with water and test it for leaks at a sink. Then they should empty the tubing.



Step 6

Using the medicine cup provided, have the students measure 10 mL of the starch solution and pour it into the open end of the dialysis tubing.

Step 7

Using another medicine cup, they should measure 10 mL of glucose solution. They should add this glucose solution to the starch solution in the dialysis tubing. Students should then squeeze all the air bubbles out of the tubing. Be sure that the students leave enough room at the open end to tie it as they did in Step 5. They should note the color of the starch-glucose solution in the dialysis tubing and record their observations in Data Table #1.

Step 8

Students should briefly rinse the outside of the bag under running water. They should then squeeze the bag gently to ensure that there are no leaks. If they find a bag leaking at an end, they should retie it securely.

Step 9

Next, they should completely submerge the model cell into the cup of water and starch indicator solution. They should allow osmosis and diffusion to occur for 20-30 minutes.

Step 10

After 30 minutes, they should test the water in the cup for sugar content, as they did in Step 2. They should note any color changes in the dialysis tubing and in the cup and record these observations in Data Table #1.

Note: *A positive (+) result for the presence of starch is indicated by a blue-black color of the final solution. If the starch test result is negative (-), the final solution will be an orange/brown color.*

Step 11

Be sure that the students wash their hands and clean up and dispose of any waste materials as you direct them.

Recording Observations

Data Table #1

Characteristic	Water in Cup	Solution in Tubing
Initial Color	**Yellow/Orange**	**Whitish/Colorless**
Final Color	**Yellow/Orange**	**Blue/Black**
Initial Glucose (+/-)	–	+
Final Glucose (+/-)	+	+
Initial Starch (+/-)	–	+
Final Starch (+/-)	–	+
Initial Solutes	**Starch indicator**	**Sucrose, Starch**
Final Solutes	**Starch indicator, Sucrose**	**Sucrose, Starch**
Change in volume observed	**Decrease**	**Increase - bag swelled**

Questions

1. What is simple diffusion?

   Simple diffusion is the movement of molecules from an area of high concentration to an area of low concentration. It is passive and, therefore, does not require energy.

2. What is osmosis, and how does it differ from simple diffusion?

   Osmosis is a special case of diffusion. It is the diffusion of water across a semipermeable membrane. It also requires no energy. It occurs when solutes cannot pass through the membrane, but water can.

3. What are two characteristics of substances that do not allow them to pass through the semipermeable membrane of living cells?

   Molecules which are very large (i.e. proteins) or particles that have an electrical charge (i.e. ions), do not pass easily - if at all - through the cell membrane.

4. What types of substances pass easily through living cell membranes?

   Small, uncharged particles - such as water molecules, oxygen and carbon dioxide - pass through the cell membrane easily.

5. Did the sucrose molecules pass through the dialysis tubing? How do you know?

   Yes, the sucrose did pass through the tubing. The glucose test strip gave a positive result for sucrose in the water in the cup at the end of the experiment.

6. Did the starch molecules pass through the dialysis tubing? What evidence do you have to support this conclusion?

   No, the starch molecules did not pass through the tubing. The starch indicator turned the starch inside the tube a dark blue-black color. The fluid in the cup did not turn color, so no starch was present.

7. Why did we use dialysis tubing as a model for a cell membrane?

   Because it was semipermeable. It let water and sucrose through, but didn't permit the starch molecules to diffuse out into the cup.

8. Did osmosis occur with respect to the model cell? What observation led you to this conclusion?

   Yes, osmosis occurred. The size of the dialysis bag increased during the experiment. The bag swelled up because the starch molecules were trapped inside and water moved into the bag (i.e. down its concentration gradient) by osmosis.

9. Why were the results for final starch and final sucrose filled in already in Data Table #1? (**Hint:** Would all of the solutes diffuse out of the dialysis bag? Why or why not?)

   These results were already filled in because the contents of the bag would only diffuse out of the bag - if they could - until the concentration outside the bag was the same as inside the bag. Therefore, not all the sucrose or starch molecules would have left the bag.

Assessment

Assign a short research and writing project dealing with how osmosis effects human cells. You can introduce the topics of hypertonic, hypotonic, and isotonic solutions. Let the students decide what would happen to body cells placed in each type of solution.

Have your students research the topic of osmotic pressure. Why doesn't osmosis continue across a membrane until the concentration of solutes on either side is equal? In other words, ask them why at some point does osmosis have to stop?

Our body cells need a constant supply of oxygen and nutrients in order to grow and sustain life. They are also continuously producing carbon dioxide and metabolic wastes that need to be removed. Using the knowledge gathered in this investigation, explain how these materials get into and out of a cell.

Explain how water passes from the roots of a plant all the way to its highest point.

How does the water that we drink get into our cells from the gastrointestinal tract?

Cross-Curriculum Integration

Assign the students an illustration project. Have them look up the chemical formula for glucose, and draw it as a dashed-line molecular model. Have them compare this to the average molecular weight for starch. They can draw several repeating sugar units in a starch molecule to give them an appreciation for how large it can be.

Human Biology/Health
Relate to students how blood dialysis machines work and how the process is similar to this investigation.

Neo/SCI
Student's
Guide

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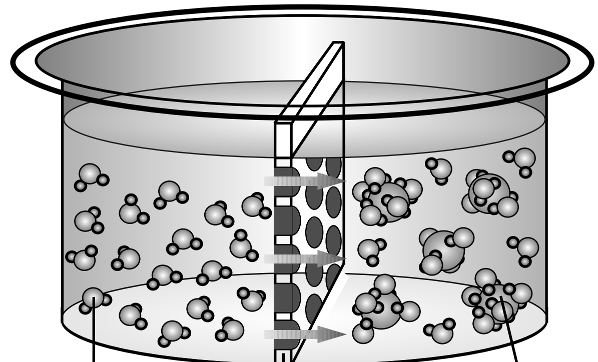
Osmosis Simulation Activity Model

Objectives

- ***Demonstrate*** **and observe the process of osmosis between hypotonic and hypertonic solutions separated by a semipermeable membrane.**
- ***Study*** **the effects of solute concentration, temperature, and pH on osmosis.**
- ***Predict*** **the characteristics of substances which allow them to pass through a semipermeable membrane.**
- ***Explain*** **the causes and effects of water movement in living cells.**

Background

The plasma membrane is made up of a variety of different organic molecules. "Phospholipids" are te most common component. Phospholipid molecules have a dual nature. One end of the molecule is polar (the phosphate head) and the other is non-polar (the lipid tail). The phospholipid molecules in a cell membrane line up in two adjacent layers, much like two pieces of bread in a sandwich. The polar phosphate groups arrange themselves on the outside of the sandwich, while the non-polar lipid tails are found in the center of the sandwich. Since water is a polar molecule, it has a slight negative charge at one end and a slight positive charge at the other end, and is attracted to these polar phosphate groups. The lipid tails, which do not mix well with water - much like salad oil in water - are hidden on the inside of the membrane and do not have to interact with the polar water molecules. This arrangement allows the cell membrane to mix well with the fluids, which are mostly water, both outside and inside the cell. The fluid outside the cell is called "extracellular," and the fluid inside the cell is called "intracellular" or "cytosol."



The addition of a solute (sucrose) to one side of a semipermeable membrane reduces the number of water molecules that can more freely on that side. It is because the water molecules bind to the solute molecule increasing its physical size, thus making it more difficult to move around. Water then moves by osmosis from the area where water molecules are more concentrated to an area that is less concentrated.

The cell membrane has several components besides phospholipids, including proteins which act as channels through the membrane, cholesterol molecules, enzymes, and carbohydrates. The cell membrane is said to be "selectively permeable." That is, it lets some substances pass freely into and out of the cell, but excludes others altogether. Molecules which are large - such as proteins and starch - and charged particles - such as ions, are usually not able to get through the cell membrane unless the appropriate "protein gate" is present. Molecules which cannot pass from one side of the membrane to the other can become concentrated on one side of the membrane.

Remember that a solution is a mixture of different types of molecules in a liquid. One of the molecule types acts as the solvent - the substance in which the other substance is dissolved. The molecule which is dissolved in the solvent is called the "solute". In living cells, the solvent is water. This is true for both the extracellular fluid and the cytosol. Many substances are dissolved in these aqueous solutions, including ions, proteins, waste products such as urea, and nutrient molecules such as glucose.

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Recall, also, that diffusion occurs whenever a concentration gradient exists between two areas and nothing blocks the diffusing substance from spreading out through that area. The cell membrane, however, acts as a barrier to certain types of molecules. If a solution becomes more concentrated on one side of a semipermeable membrane than on the other, osmosis will occur. Osmosis is the passive (i.e. no energy) diffusion of water molecules down their concentration gradient (i.e. from high concentration to low) across a semipermeable membrane. In other words, if the extracellular fluid solutes become more concentrated than the solutes in the cytosol, water will leave the cell by osmosis. Water is moving down its own concentration gradient. The solution outside the cell in this case is said to be "hypertonic" to the cytosol, meaning that it has a greater concentration of solutes than does the cytosol. In animal cells, this process is called "crenation," and can lead to the shrinkage and death of the cell. In plants, this process is called "plasmolysis," and can also lead to the death of the plant cell.

The opposite process, also involving osmosis, can occur if the intracellular fluid (i.e. cytosol) becomes more concentrated than the extracellular fluid. In this case, the concentration of water is greater outside the cell, so water moves across the membrane down its concentration gradient. The extracellular fluid in this case is said to be "hypotonic" to the intracellular fluid. The movement of water into the cell can lead to the rupture of the cell membrane in animal cells, a process called "lysis". In plant cells, this pressure within the cell pushes the plant cell membrane tightly against the cell wall, firming the cell. This pressure is called "turgor pressure", and the plant cell is said to be "turgid".

The ideal situation for animal cells is to have the intracellular and extracellular fluid in osmotic balance with one another. In such a case, the solutions would be isotonic (i.e. having the same solute concentrations). Here, water diffuses passively across the membrane at the same rate in both directions so there is no net gain or loss of water from the cell. This process of keeping solutes and water balanced is called "osmoregulation". Most organisms have some method of regulating their water balance, from the cellular level to specialized organs (i.e. like your kidneys) which help do this job for the entire organism.

The hydrostatic (osmotic) pressure created between a hypotonic and a hypertonic solution separated by semipermeable membrane can be expressed by the following formula:

$$P \text{ (osmotic pressure)} = K \times T \times \frac{C}{M}$$

Where:
K is a factor for the solvent. For water K=1.86°C. This value corresponds to the lowering of the freezing point for one mole of solute in a liter of water.

C is the concentration of the solute in the hypertonic solution (solution in osmometer vessel)

T is the temperature in °K. An increase in temperature increases the speed of the molecules and therefore their diffusion.

M is the molecular weight of the solute.

Time Requirements

30-45 minutes

Safety & Disposal

You should always wear safety goggles, gloves and a lab apron to protect your eyes and clothing when working with any chemicals. Be sure that you wash your hands before leaving the laboratory.

Neo/SCI Student's Guide

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Activity 1

Observing and quantifying osmosis

What you need

Osmosis Simulation Activity Model
Hypertonic solutions
- Colored sucrose solution, 1M
- Colored sucrose solution, 0.5M *
- Colored acidic sucrose solution, 1M *
- Colored basic sucrose solution, 1M *

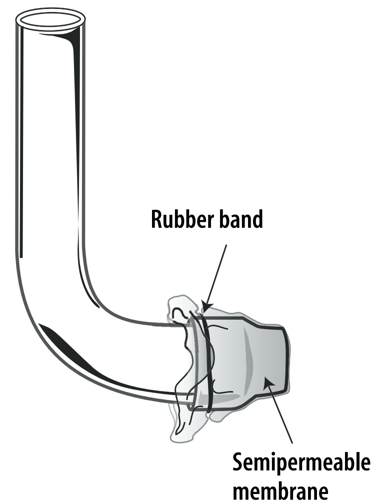
Distilled water
Rubber band
1 Ruler
1 Semipermeable membrane sheet

** optional*

What to do...

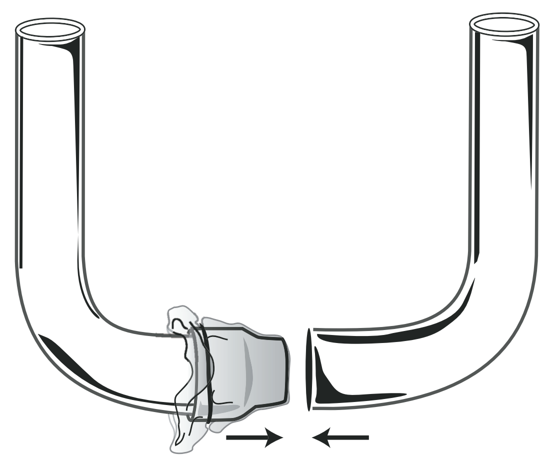
Step 1

Place a wet piece of semipermeable membrane over the L-shaped tube and hold it in place by wrapping a rubber band around it.



Step 2

Fit the L-shaped tube with the semipermeable membrane attached, into the other L-shaped tube. As an option, you may want to lightly grease the connector to ensure that the unit is watertight.



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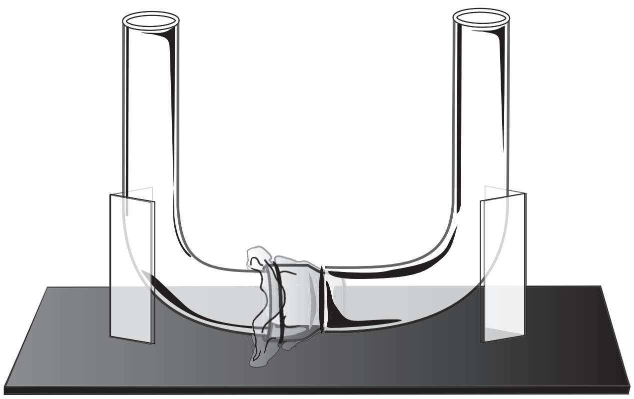
Step 3

Place the assembled apparatus on the stand as shown in the illustration below.



Step 4

Pour the prepared, colored sucrose solution (1M) into one side of the apparatus and fill it to the top with a slight overflow.

Step 5

Caution: *Use extreme caution when inserting the glass capillary tube into the rubber stopper. Lubricate the stopper and the glass tubing. Use heavy leather gloves to protect your hands from shattering glass. To prevent puncture wounds, be sure your hand is clear of the hole. Do not push the capillary tube all the way through the hole of the rubber stopper. For safety reasons, your teacher may opt to perform this step for you. Carefully insert the glass capillary tube half way into the one-hole rubber tubing.*

Carefully insert the capillary tube into the rubber stopper. You may apply a thin layer of petroleum jelly around the rubber stopper hole to make it watertight.

Step 6

Insert the rubber stopper with the capillary tube attached to it, into the top orifice of the filled side. Twist it half a turn to ensure that it is securely placed into the orifice and that the solution is seen near the bottom end of the capillary measuring tube.

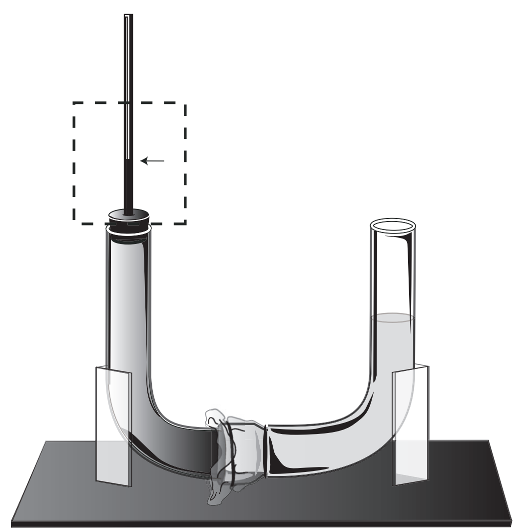
IMPORTANT. *Make sure that the hypertonic solution is touching the rubber stopper and that there are no air bubbles or air gaps between the solution surface and the bottom of the rubber stopper. Add additional solution if necessary.*

Step 7

Fill the other side of the osmosis apparatus 1/2 full with distilled or tap water.

Step 8

Note the starting level of the solution in the capillary tube and record it in Table 1.
Start your timer.



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Step 9

Over time, the level of the hypertonic colored solution should rise in the vertical capillary tube. Record the position of the colored solution every 2 minutes for up to 30 minutes and record your results in Table 1.

Step 10

As an option, your teacher may ask you to repeat the experiment, using 0.5 M sucrose solution, acidic sucrose solution, basic sucrose solution or solutions of different temperatures or other solutions of your choice including starch and salt.

Step 11

Wash your hands and clean up you area as your teacher directs you.

Table 1
Sample Results

Movement in mm				
Time	Sucrose,1M	Sucrose, 0.5 M	Sucrose, 1 M plus HCl	Sucrose, 1 M plus NaOH
0				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				
22				
24				
26				
28				
30				

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Questions

1. Did osmosis occur through the semipermeable membrane? What observation led you to this conclusion?

 ....

 ....

2. What is osmosis, and how does it differ from simple diffusion?

 ....

 ....

 ....

3. What are two characteristics of substances that do not allow them to pass through the semipermeable membrane of living cells?

 ....

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4. What types of substances pass easily through living cell membranes?

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Going Further

Using the formula provided in the "Background" section, calculate the osmotic pressures of various solutes of varying concentration and temperatures.

Design experiments to test the permeability of various types of membranes, both synthetic and natural such as animal bladder, etc.

Design experiments to test the osmotic pressure of other solutes such as caffeine, nicotine, etc. under varying environmental conditions.

You can observe osmosis in living plant cells. Use a red onion from the grocery store for this experiment. Peel off the thin skin from the inside of one of the onion's leaves. Place this tissue into a drop or two of distilled water on a microscope slide and cover it with a coverslip. Observe the cells under the compound light microscope. Now, add several drops of 5% saline solution on one side of the coverslip and wick it under the coverslip using a paper towel. Watch what happens to the onion cells. Since the salt in the extracellular fluid is not allowed through the cell membrane, water from the cells moves out (i.e. down its concentration gradient). This process in plant cells is called "plasmolysis". Re-flood the slide with distilled water. What happens? Why?

Predict the effect of exposing model cells to hypertonic, hypotonic, and isotonic solutions. Determine the water potential (i.e. movement of water in and out of a cell) of potato cells. Use a cork borer to cut a potato into cylinders. Place them in various sucrose solutions and calculate the change in mass over time. Determine what will happen if the potato cylinders are placed in a hypertonic, hypotonic, and isotonic solutions.

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New ideas for teaching science

Learn and Read More About It

Benjamin, Clinton; Garman, Gregory; and Funston, James. *Human Biology*. McGraw-Hill. 1997.

Allan J. Tobin and Richard E. Morel. *Asking About Cells*. HBJ School Div. 1997

Andres Llamas Ruiz and Luis Rizo. *The Life of a Cell (Cycles of Life)*. Sterling Publications. 1997

Neat Websites

Information on biology-related topics in easy-to-understand format for students
http://www.chem4kids.com/biology4kids/cell/lys.html
http://www.chem4kids.com/biology4kids/chem/prot.html
http://esg-www.mit.edu:8001/esgbio/cb/membranes/transport.html

Provides a thorough overview of cell structure and function
http://www.kapili.com/biology4kids/cell/index.html

Provides background information and suggested activities to learn more about cells
http://raven.umnh.utah.edu/secondlevel/teen/teen.html

Provides a wide selection on various types of cells and related information
http://www.cellsalive.com/