The Young Scientist Program
Diffusion and Membrane Permeability Teaching Kit
Teacher Handout

Washington University Medical School

Funding provided by The Leon Lowenstein Foundation

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Timing: This lesson is expected to take up to an hour long class period, with the final gummy bear experiment completed during the following class. If the class is running long, the gummy bear can be set up overnight and the iodine/starch experiment can be run the following day.

DVD: The DVD included in this kit is meant to demonstrate the experiment and provide some suggestions on how to make the demo more successful. It will also provide some background information that can serve as a guide for teaching the students the core concepts in this exercise.

Curriculum Links:

Missouri Science Standards

Biology: http://dese.mo.gov/divimprove/curriculum/GLE/documents/cur-sc-bio-cle-1108.pdf
Non-biology: http://dese.mo.gov/divimprove/curriculum/GLE/documents/cur-sc-other-cle-1107.pdf

Biology

Strand 1-1A: Objects, and the materials they are made of, have properties that can be used to describe and classify them.

Strand 3-2-Ac: The cell contains a set of structures called organelles that interact to carry out life processes through physical and chemical means (cell membrane).

***Strand 3-2-F (most relevant):** Cellular activities and responses can maintain stability internally while external conditions are changing (homeostasis).

Strand 7-1-A: Scientific inquiry includes the ability of students to formulate a testable question and explanation, and to select appropriate investigative methods in order to obtain evidence relevant to the explanation.

Strand 7-1-C: Scientific inquiry includes evaluation of explanations (laws/principles, theories/models) in light of evidence (data) and scientific principles (understandings).

Chemistry

Strand 1-Bc: Predict the effects of solvent and solute polarity on solubility ("like dissolves like"); and predict the effects of temperature, surface area, particle size, and agitation on rates of solubility.

Next Generation Science Standards

http://63960de18916c597c345-8e3bed018cb857642bed25a591e65353.r63.cf1.rackcdn.com/Standards%20by%20DCI%20FINAL%20with%20TOC%20and%20page%20number.pdf

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Middle School

MS-PS1-4: Develop a model that predicts and describes changes in particle motion, temperature, and state of a pure substance when thermal energy is added or removed.

MS-LS1-2: Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function (cell membrane).

MS-ETS1-1: Define the criteria and constraints of a design problem with sufficient precision to ensure a successful solution, taking into account relevant scientific principles and potential impacts on people and the natural environment that may limit possible solutions.

High School

HS-PS1-5: Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.

Goals: There are three main take home messages we hope the students come away with. Before the hands on portion, take some time to discuss what they will be doing, and why it is valuable. Please refer to the enclosed DVD for some suggestions on how this conversation might go, and a demonstration of the experiment.

1. DIFFUSION is a passive (requires no energy), spontaneous (happens on its own) process involving the random movement of particles or molecules from an area of high concentration to an area of low concentration.

2. EQUILIBRIUM across a membrane occurs when the number of molecules is even on both sides, and there is no longer a concentration gradient.

3. SELECTIVE PERMEABILITY across a membrane can result in differing salt concentrations on either side of the membrane and will cause movement of water towards the side with higher salt concentration.

These are chemistry concepts that will be taught in the context of biological systems. The movement of molecules across cellular membranes controls many biological processes including signal transduction, cell motility, muscle contraction, neural transmission, intercellular communication and many more. You should try to emphasize the biological importance of this lesson, despite its basis in chemistry and the use of abstract experiments. In addition to the specific concepts covered by this lesson, it will also hopefully help student to appreciate the relationship between chemistry and biology.

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Tell the students that we will be learning about diffusion and equilibrium across membranes. The following questions can be used to get the students thinking about the concepts that will be covered in this lesson. Collect several answers from the class for each question and write them on the board. At the end of the lesson you can return to these questions and ask the students which answer they now think is correct, or if a new answer is appropriate based on what they learned.
A few questions to start:

1. **What causes food coloring to spread out when dropped into a glass of water?**
   Food coloring molecules will spread out evenly into the larger volume of water through the process of diffusion because molecules will attempt to reach an equal concentration throughout the glass.

2. **Why do you think Gatorade is better to drink than water when a person is dehydrated?**
   Gatorade contains molecules that help maintain the proper balance of salt and water on the inside and outside of a person's cells. Maintaining this balance helps cells uptake water.

3. **Why do plants wilt when dry and why do they become rigid when fully watered?**
   When plant cells uptake water the cells expand and become rigid, while the loss of water causes the cells to shrivel.

4. **Why do your fingers prune after taking a bath?**
   Bath water has a lower salt concentration than your body, so the water moves into your cells to reach equilibrium, causing them to swell and bloat.

Class Experiments:

Materials (per group):
Two cups
Ziploc bag
Tap water
Eppendorf tube containing corn starch
Two gummy bears
15ml tube containing iodine
Food coloring
Dropper

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Heating block/microwave
Lab tape
Gloves
Timer
You should have two beakers of water pre-heated before class to steaming (not boiling) with either the included heating block or a microwave. They will be used for multiple experiments.

Safety:
For the walking around the classroom experiment there ensure that students are walking safely. DO NOT CHOOSE STUDENTS TO PARTICIPATE WHO ARE LIKELY TO START FIGHTS. Students must not drink the iodine! Gummy bears should not be eaten if they have been used in an experiment, but if there are extra unused within the bag they can be consumed. Students should wear gloves for handling the dialysis tubing. Steaming water can burn, students should be very careful handling it during the experiments.

Diffusion – estimated time 5 minutes (Call on students to read sentences from intro)

Materials:
Two cups
Tap water
Heating block/microwave
Steaming water
Food coloring
Dropper
Timer

One important physics concept that our cells constantly use in order function properly is diffusion. This section in the lesson will focus on demonstrating the general concept of diffusion. Diffusion is the random movement of particles or molecules from an area of high concentration to an area of low concentration. No energy is required and the process occurs spontaneously. This is illustrated in the picture below. Particles will diffuse until they are evenly distributed, or in other words they will diffuse until equilibrium is reached.

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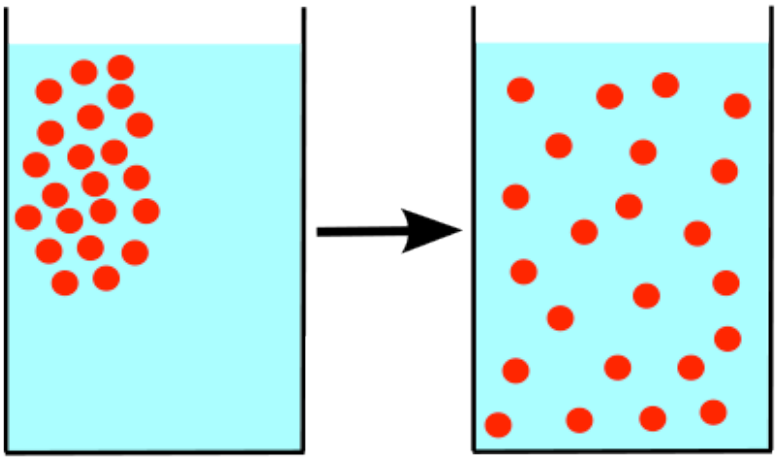

Experimental Procedure and Discussion Questions

The concept of diffusion can be demonstrated with a simple drop of food coloring in a container of water.

1. Have each group take out both cups, a tube of food coloring and a dropper. Have a member of each group come line up with one cup and add heated water up to the line (about 200ml). Another student from the group should fill the other cup with cool tap water.

2. Have students from each group drop 2 drops of food coloring into each cup of water at the same time.

3. Instruct the class to observe what happens to the drop of food coloring. Be sure not to stir the water. One student in each group should start the stopwatch when the food coloring is dropped, and other students should record the time that it takes for the food coloring in each cup to become evenly distributed throughout the cup. The drop should spread out from a single concentrated drop of color to eventually evenly color the entire container of water. This will happen rapidly at first and then slow down. Ask the class to raise their hand for whether the coloring in the hot or cold water diffused faster, or if they took approximately the same time.

This is diffusion at work right before your eyes! The tiny colored molecules that make up the food coloring diffuse through the water from an area where they were highly concentrated to an area where there was previously no color.

Q: A: Describe what happened to the food coloring after being dropped into the water.
It spreads out throughout the volume of the water.

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B: Does the speed of the color spreading out change over time? If so, why?
The food coloring should at first spread very fast and then begin to slow down.
C: Can you think of something you could add to the cup to prevent the color from diffusing throughout the entire cup?
Place a barrier of some kind in the cup to block the spread of food coloring to the other side.
D: Does the hot water make diffusion occur faster or slower? What do you think would happen in ice cold water?
Hot water should increase the speed of diffusion; cold water should decrease the speed.

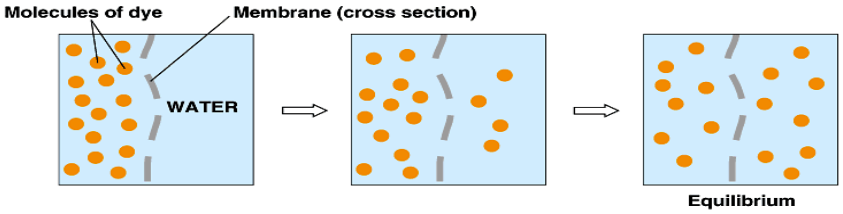
References for more info:
Diffusion Overview: http://en.wikipedia.org/wiki/Diffusion
All Topics Reviewed: http://www.biologycorner.com/bio1/diffusion.html
Video of Diffusion and Temperature: http://www.youtube.com/watch?v=STLAJH7_zkY
Lesson Plan: http://en.wikiversity.org/wiki/High_School_Biology/Lessons/Lesson_3

Equilibrium – estimated time 10-15 minutes (Call on students to read sentences from intro)

Materials:
6-20 students
Lab tape or arranged desks/chairs
Timer or classroom clock to count time

Two of the most fundamental concepts that drive cell function in your body are the ideas of **equilibrium** and **concentration gradient**. These concepts are the simplest ways in which cells in the body have the potential to do work that help them survive. A solution is when a type of molecule is mixed or dissolved in a solvent (like salt or sugar dissolved in water). To set up a concentration gradient you need at least 2 solutions and a sort of barrier (**semi-permeable membrane)** that allows molecules of a certain size to cross freely. For example, Solution A is a cup of water with 100 molecules of salt, while Solution B is a cup of water with 0 molecules of salt; Solution A is more concentrated than Solution B. Now imagine that both solutions are poured into separate halves of the same cup and are divided in the middle by a membrane, which only allows molecules of a certain size to pass across. This sets up a **concentration gradient,** where salt molecules are more packed together on one side of the membrane compared to the other. A chemical property called **equilibrium** dictates that the molecules will want to diffuse in a direction across the membrane such that over

time, there is the same amount on both sides. So Solution A's salt molecules will want to move to the other side to Solution B's space, where it is less crowded with salt, and eventually there will be 50 molecules of salt in Solution A and 50 molecules in solution B. At this point, because there is no more difference in salt concentration between the two sides, the concentration gradient does not exist anymore. This situation is illustrated in the picture below.



Experimental Procedure and Discussion Questions

For this experiment, we will use students to demonstrate the concepts of concentration gradient and equilibrium.

1. Set up the classroom such that you physically divide up the class in half by arranging a row of chairs/desks or the included lab tape down the middle of the classroom. However, leave enough room between chairs/desks such that a student can walk through. **Make sure both sides are equally clear of obstacles, but choose a space in which the students will be sufficiently crowded.**

2. Place 6-20 students (depending on room size, but an even number is required) on one side of the room. Ask the students to extend out their arms and walk (slowly) around their half of the room **without bumping into each other** for 30 sec, trying to remain as spread out and far away from each other as possible. Tell them they do not have the option of crossing over to the other side. This is like a concentration gradient because one side of the room is more concentrated with students than the other side.

Q: A. Was it easy to move around without bumping into each other?

There should not be much room for free movement

B. Would it be easier to walk around if you were able to use the entire room?

Yes, of course.

C. If given a choice, how many students should be crossing over to the other side, such that it is equally easy on both sides of the room to walk around, arms extended, without bumping into each other?

The specific answer depends on how many students participate, but it should be half of the total number of participating students to create equal numbers on each side. This is an equilibrium.

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3. Start the students on the same side, but now tell the students they have the option of crossing over to either side as they wish in order to spread out as much as possible. This time give them about 60sec, and it may be helpful to stop them when you observe equal numbers on both sides of the room.

Q: C. What happened to the crowd initially when you were allowed to move around the entire room?

Students should spread quickly into the newly available area.

D. When time expired, were there a similar amount of students on both sides of the room? Was this expected?

There should be approximately even number of students on each side; this is expected in order to maximize the free space available.

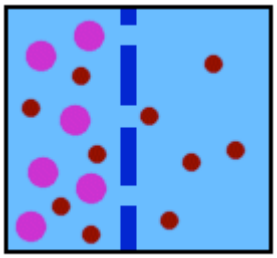
E. Beginning from an even number of students on each side, if 2 students moved to the opposite side of the room, would you still be at equilibrium? What would need to happen to restore equilibrium?

The movement of two students to the opposite side would disrupt equilibrium; the corresponding movement of two students away from the opposite side is necessary to restore equilibrium.

Molecules are constantly in motion and cross one barrier, come back, and cross over. Just because there is equilibrium doesn't mean the molecules have stopped moving.

References for more info:
General overview: http://en.wikipedia.org/wiki/Equilibrium
Video (goes a bit more detailed): http://www.youtube.com/watch?v=Gwn9oRvYiu4

Selective Permeability/Tonicity – estimated time 15-20min (plus overnight) (Call on students to read sentences from next 2 paragraphs)



Materials:
One cup
Steaming hot water
Heating block/microwave
Eppendorf tube containing corn starch
15ml tube containing iodine
Ziploc bag
Straw for stirring

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[for teacher demo:]
One extra cup
3ml iodine solution
Eppendorf tube containing corn starch
Tap water

Cells are surrounded by a membrane that keeps cell material in and unwanted material out. The cell membrane only allows movement of certain materials across, making it **selectively permeable.** Selective permeability allows our cells to maintain proper concentrations of needed molecules and ions by controlling what is allowed to enter or exit the cell. Cell membranes may restrict movement by requiring a specific electric charge, size, or interaction with proteins on the cell surface.

Experimental Procedure and Discussion Questions

In this exercise, we will explore examples of selective permeability and its effect on tonicity.

In our experiment we will be demonstrating selective permeability based on size selection. A Ziploc bag containing iodine will be placed into a cup of water containing dissolved/suspended corn starch. Starch is a large molecule containing many atoms while iodine is a much smaller molecule. Starch turns purple when it comes in contact with iodine, so we will be able to determine the presence of starch and iodine both outside and inside the bag by color indication.

Have a large beaker of water preheated to slightly hot (should just begin to steam) set up before class/the experiment utilizing the heating block provided in the kit or a microwave, this experiment will not work in the required time without steaming water.

Tell students the bag is a selectively permeable membrane, and explain that starch + iodine = purple. Drop some iodine on some dry starch to demonstrate the color change to the students.

1. Each group should have a Ziploc bag and a 15ml tube containing iodine. Instruct them to pour the iodine into the bag and close it tightly, ensuring that the bag is fully sealed before use. Have one member from each group bring their cup to the front of the room. Fill the cup up to the line with hot water.
2. They should then add cornstarch (in small eppendorf tube) to the water and mix until the starch is evenly distributed and dissolved. (Note: most of the starch will remain in suspension and not actually dissolve, so the solution should remain cloudy).
3. They should place the bag of iodine in the starch water and wait a few minutes, occasionally tapping(not swirling) the cup to stir.

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The class should answer the following question while waiting for the bag to turn purple.

Q: A. Do you think the liquid will turn purple? Where, inside or outside the bag?

The liquid should turn purple outside the bag only due to the selective movement of iodine but not starch across the plastic.

B. What possible factors may affect the speed of the reaction?

Temperature, iodine concentration, corn starch concentration, and thickness of the plastic bag are a few examples, but there may also be additional answers.

Note: The reaction will take anytime between 2 and 20+ minutes depending on these factors. If purple appears quickly, tell the students to continue observing the cup as they continue the lesson so that they can observe the reaction to completion.

After the experiment:

Q: C. What did you observe inside the bag and outside the bag?

Purple color should diffuse away from the bag into the surrounding starch water outside the bag while the liquid inside the bag should remain yellow.

D. Of the starch and the iodine, which is able to pass through the membrane (plastic bag) and what is not allowed to pass through? Why?

Only iodine can move across the membrane, while starch cannot. Starch molecules are too large to fit through the pores in the bag, while iodine can freely flow across.

E: Based on your knowledge of the selective permeability of starch and iodine, explain the reason for the differences in color inside and outside the bag.

Iodine moves out of the bag, causing the starch to turn purple; because the starch cannot enter the bag the liquid inside the bag does not turn purple and remains yellow.

(Have class read these paragraphs)

Similar to other molecules such as proteins and salts, water also moves across a membrane down its concentration gradient. The movement of water across a cell membrane is known as **osmosis.** Selective permeability can result in an increased concentration of larger molecules such as proteins and sugars on one side of the membrane since they are too large to pass through. Because the concentration of large molecules is higher on one side of the membrane, the relative concentration of large molecules on the other side is less and the overall solution is not at equilibrium. This will cause the influx of water from the less concentrated side over to the more concentrated side in order to equilibrate the concentration of the salt on both sides of the membrane.

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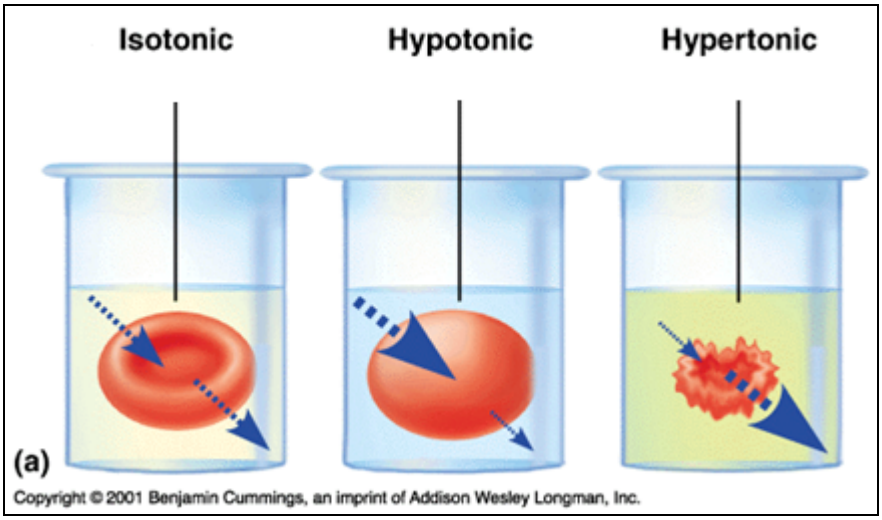
If the solution outside a cell has less solute (ions, salt particles, etc) than inside the cell the solution is known as a **hypotonic solution**, while a solution with more solute than the cell is a **hypertonic solution.** A solution of equal solute concentration to the inside of the cell is an **isotonic solution**. A hypotonic solution will cause the cell to swell as water enters to move down its concentration gradient while a hypertonic solution will cause the cell to shrivel as water leaves.



4. We will now use gummy bears to simulate the effect of a cell placed in a hypotonic solution. This will require overnight incubation in water to observe the full effect. Each group should place one gummy bear in a cup of cool tap water and another gummy bear in an empty cup for comparison. Use COLD tap water for this experiment, warm water will dissolve away the gummy bear.

Materials:
Two cups
Cool tap water
Two gummy bears

Q: D: What do you think will happen to the gummy bear in water?
They will swell, resulting in an increase in size along with a bloated, fragile appearance.

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The next class, have students slowly pour out the water so that size comparison is not affected by diffraction of light by the water.

Q: E: What happened to the gummy bear? Did it grow or shrink, and did its appearance change? What caused this change in size?

The gummy bear will increase in size due to an overall influx of water into the gummy bear.

F: Is the water hypotonic, hypertonic or isotonic relative to the inside of the gummy bear? How does this determine the direction of water flow?

The water is hypotonic. Water is therefore less concentrated inside the gummy bear and therefore moves down its concentration gradient, resulting in an overall influx of water into the bear.

G: What do you think would happen if the gummy bear was placed instead in very salty water? Why?

The gummy bear would shrivel; the salt water would be hypertonic, causing water to leave the bear and an overall decrease in size.

Teacher's note: The salt water experiment was not included in the experimental procedure because it proved highly variable and required extremely high salt concentrations since the inside of gummy bears are already pretty concentrated with salt/sugars. If you wish to include this and then discuss with the class the reasons for troubleshooting, that is also possible.

References for more info:

Semipermeable membranes (a bit detailed): http://en.wikipedia.org/wiki/Selective_permeability
Semipermeable membrane slideshow lecture: http://www.youtube.com/watch?v=Ya9Vg46ZBPA
Tonicity (good description): http://en.wikipedia.org/wiki/Tonicity
Tonicity (another good overview) http://www.absoluteastronomy.com/topics/Tonicity
Video on iodine experiment: http://www.youtube.com/watch?v=DFhlqgMy1Dk&feature=fvwrel
Tonicity mini slideshow lecture: http://www.youtube.com/watch?v=_slUL3kMZlU&feature=related

Conclusions

Return to the questions from the beginning of the lesson, have the answers changed?

1. **What causes food coloring to spread out when dropped into a glass of water?**
   Food coloring molecules will spread out evenly into the larger volume of water through the process of diffusion because molecules will attempt to reach an equal concentration throughout the glass.

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2. **Why do you think Gatorade is better to drink than water when a person is dehydrated?**
   Gatorade contains molecules that help maintain the proper balance of salt and water on the inside and outside of a person's cells. Maintaining this balance helps cells uptake water.

3. **Why do plants wilt when dry and why do they become rigid when fully watered?**
   When plant cells uptake water the cells expand and become rigid, while the loss of water causes the cells to shrivel.

4. **Why do your fingers prune after taking a bath?**
   Bath water has a lower salt concentration than your body, so the water moves into your cells to reach equilibrium, causing them to swell and bloat.

Final Evaluation
Fill in the blank…. (use concentration gradient, diffuses, equilibrium, into, out of, increase, shrink, water, selectively permeable, hypertonic, isotonic, hypotonic, osmosis).

The passage below will utilize all vocabulary learned throughout the lesson to explain the final experiment.

When a gummy bear is placed in pure water, water flows __*into*____ the gummy bear, causing it to __increase__ in size. The surface of the gummy bear acts as a ___*selectively permeable* _____ membrane, blocking movement of large molecules and only allowing movement of small molecules such as__*water*__. The pure water solution is therefore a(n) __*hypotonic*___ solution. If the gummy bear is placed in very salty water, water moves __*out of*__ the gummy bear down its ___*concentration gradient*___, causing the bear to __*shrink*__. The water __*diffuses*__ freely across the membrane through a process known as ____*osmosis*___ until it has reached ____*equilibrium*____. The salt water is therefore a(n) ____*hypertonic*__ solution, while a solution of equal salt concentration as the inside of the gummy bear is a(n) ___*isotonic*__ solution.

Please fill out the evaluations! Afterward, please return the completed evaluations and students' responses to the critical though questions to YSP. Fax to Rochelle Smith at **314-362-3369**, or mail to YSP at 660 S Euclid Ave. Box 8226, St. Louis, MO 63110

Thanks!

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