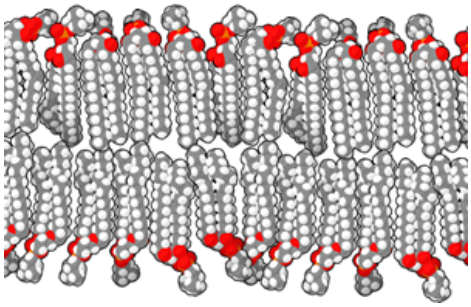
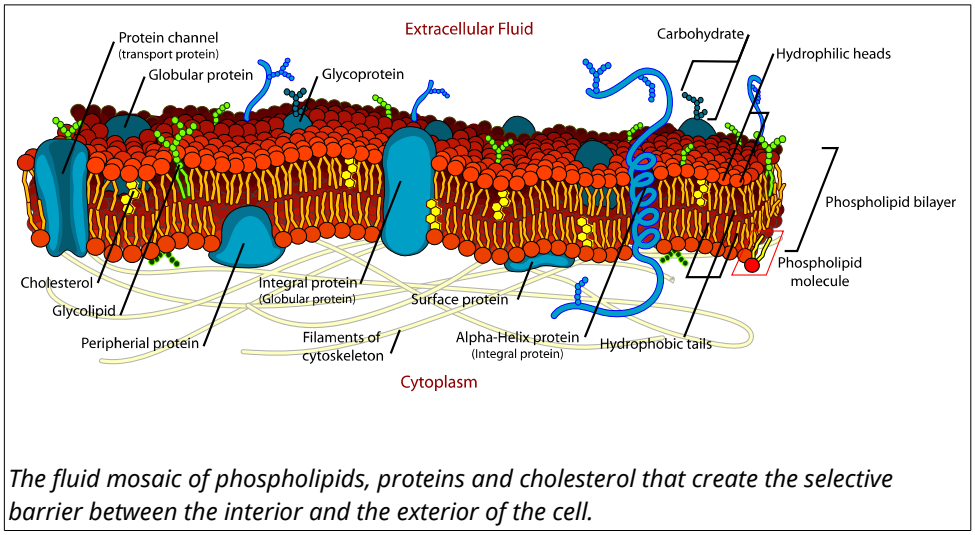
Understanding Membranes



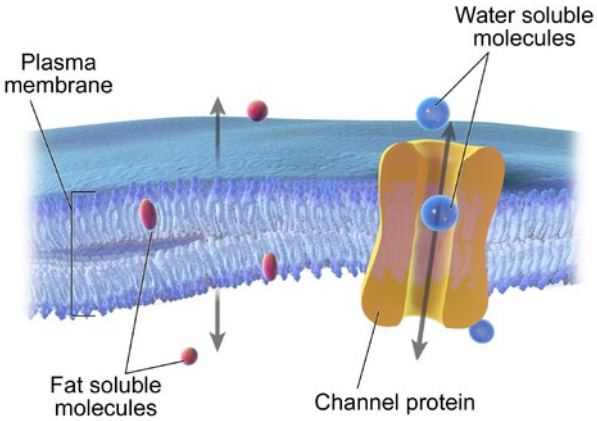
The cell membrane is the barrier that separates the cytoplasm from the external world. The cell membrane consists primarily of phospholipids in a bilayer. Phospholipids are amphipathic with a polar head (phosphate group) and a hydrophobic tail (2 hydrocarbon chains). Due to the chemical properties of the heads being attracted to water and the tails having a desire to avoid water, phospholipids self assemble into micelles. Cell membranes form from a phospholipid bilayer where the lipid tails interact with each other and the phosphate heads face the external water environment or the internal cytoplasm of the cell.

The cell membrane does not solely consist of phospholipids but also have proteins and cholesterol inserted into the bilayer. As the image of the bilayer above indicates, the molecules are constantly moving and flow in a lateral motion. Cholesterol modulates the fluidity of this motion. Proteins associated with the membrane may sit on either side (**peripheral proteins**) of the membrane or pass through both layers of the membrane (**transmembrane proteins**). The model that describes the components of the cellular membrane is referred to as the **Fluid Mosaic Model**. This model states that the cell membrane is a mosaic of 1) Phospholipids 2) Proteins 3) cholesterol that move about in a side to side motion.



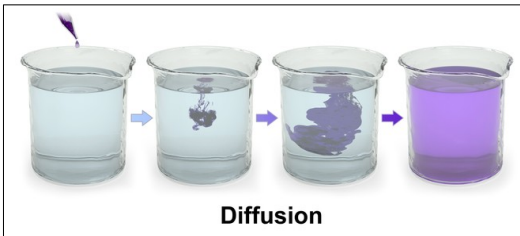
The fluid mosaic of phospholipids, proteins and cholesterol that create the selective barrier between the interior and the exterior of the cell.

Small uncharged molecules pass through the double layer of phospholipids. Polar, charged or large molecules have great difficulty passing through the membrane and require the aid of transmembrane proteins. An example of a transmembrane protein that facilitates movement of a polar substance is aquaporin, which permits the free movement of water.



Credit: BruceBlaus (CC-BY-SA 3.0)

Diffusion



Diffusion

Concentrated dye diffuses along the concentration gradient until reaching equilibrium (no net movement).
Credit: BruceBlaus (CC-BY-SA 3.0)

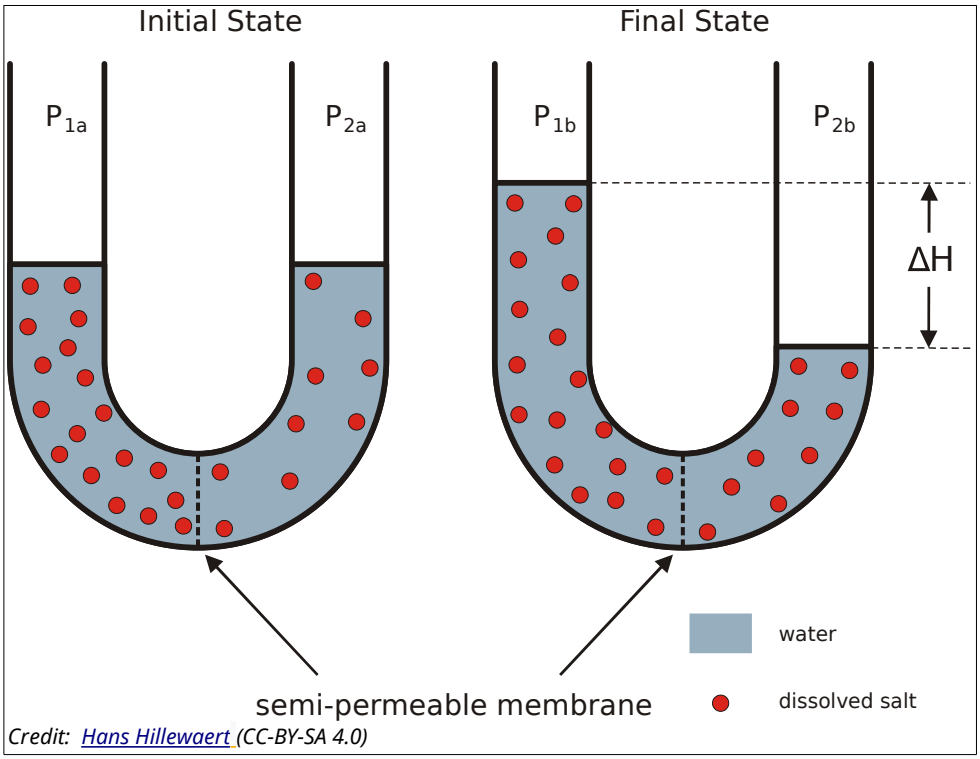
Diffusion is the net movement of a substance from high concentration to low concentration. This difference in concentration is referred to as a **concentration gradient**. This movement does not require any external energy, but uses the free energy intrinsic to the system.

- Temperature Effects on Diffusion
  - Temperature/Diffusion Simulation
- Molecular Mass Effects on Diffusion
  - Size/Mass/Diffusion Simulation

Osmosis

Osmosis is a special case of diffusion. Instead of observing the net change in solute, osmosis follows the net movement of solvent across a **semipermeable membrane**. Since a semi-permeable membrane permits specific things to pass through, some solutes are partitioned.

- Simulation of diffusion through a semipermeable membrane (CC-BY 4.0 Concord Consortium)



Credit: Hans Hillewaert (CC-BY-SA 4.0)

A cell lacking a cell wall is affected greatly by the tonicity of the environment. In a **hypertonic** solution where the concentration of dissolved solute is high, water will be drawn out of the cell. In a **hypotonic** solution where the concentration of dissolved solute is lower than the interior of the cell, the cell will be under great **osmotic pressure** from the environmental water moving in and can rupture.

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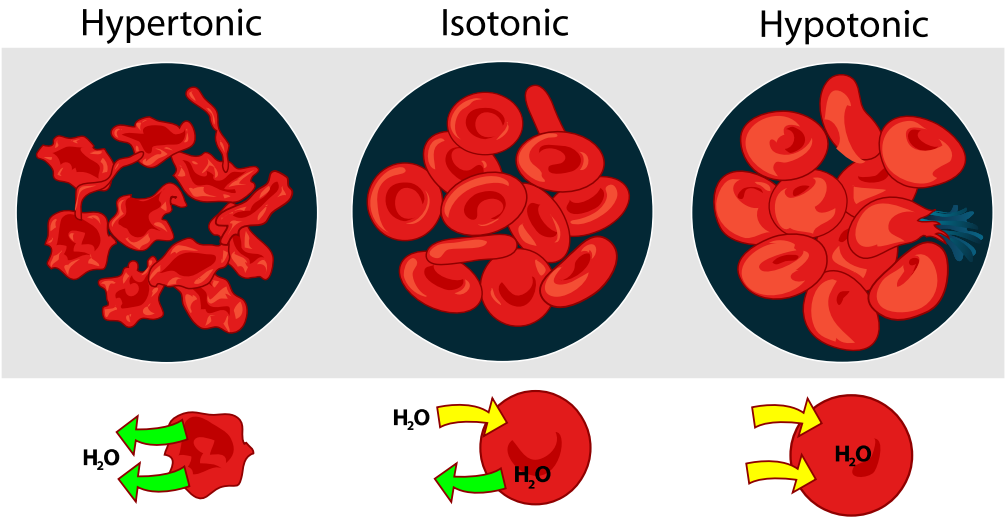
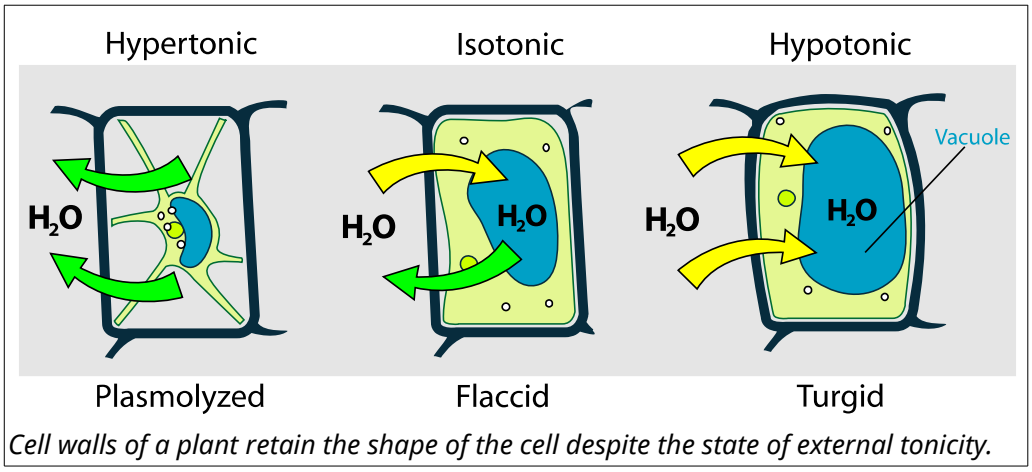


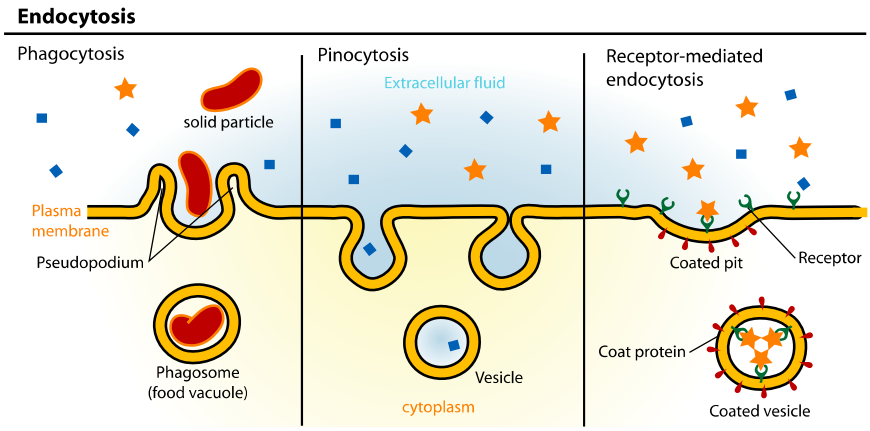
Plants have rigid cell walls composed of cellulose. These cell walls permit for maintenance of cellular integrity when the external environment is **hypotonic** (less dissolved substances). In this situation, the water moves into the cell. Without the cell wall, the cell would burst open from the excessive water pressure entering the cell. This state of swelling is referred to as turgid, resulting from **turgor pressure**.

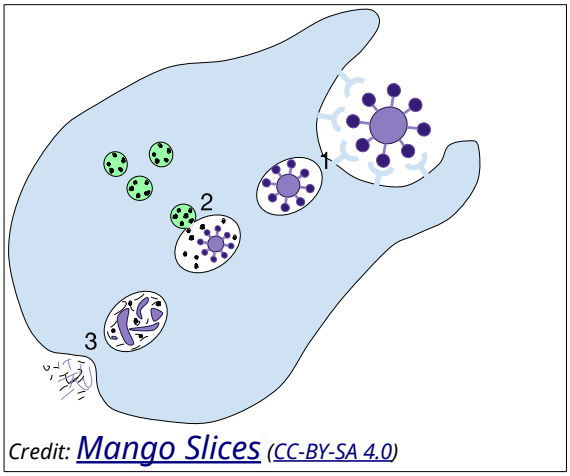


Cell walls of a plant retain the shape of the cell despite the state of external tonicity.

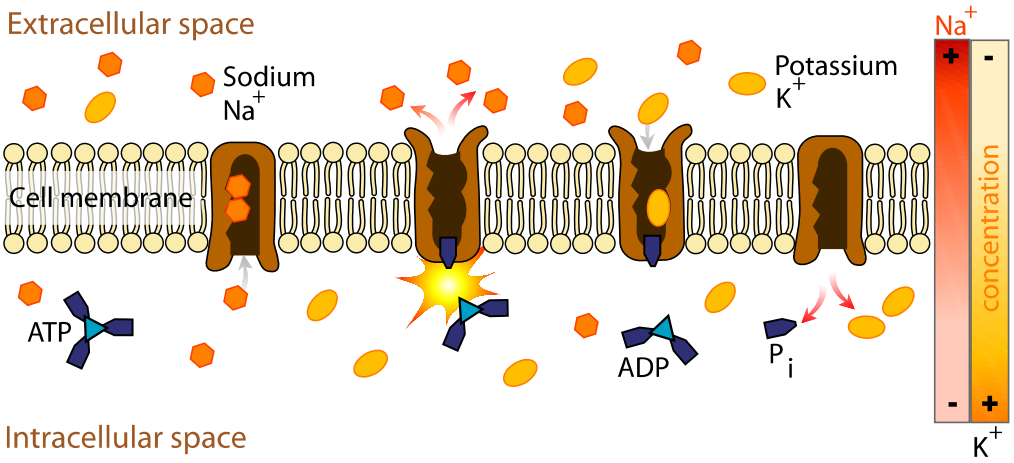
When the exterior environment is **hypertonic** , (greater amount of dissolved substances), the reverse condition occurs whereby the cellular fluid exiting the cell reduces the size of the cytoplasm. This condition is referred to as **plasmolysis**

Active Transport Mechanisms





Credit: *Mango Slices* *(CC-BY-SA 4.0)*



Further Reading

- http://www.visionlearning.com/en/library/Biology/2/Membranes-I/198/reading
- http://www.visionlearning.com/en/library/Biology/2/Membranes-II/204
- http://www.biologycorner.com/bio1/notes_diffusion.html (CC-BY-NC)

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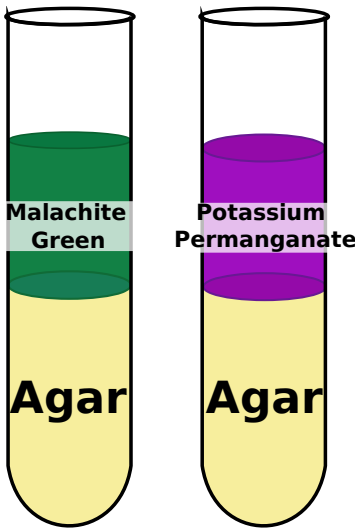
Activity: Do larger things diffuse faster?

Agar is a gelatinous substance derived from a structural carbohydrate found in seaweed. It is often used in cooking as a vegetarian alternative to gelatin and can be used as a thickener. Microbiologists pour plates of agar containing nutrients in order to isolate and grow bacteria and other microbes. As with gelatin, the long fibery nature of this structural carbohydrate permits it to be melted and tangled together in a mesh-like network where the spaces between molecules are filled with solution. Altering the amount of fluid solution will change the pores between fibers. More fluid will create a looser gel that has larger spaces between molecules. Reducing the fluid solution volume will conversely create a stiffer gel with smaller spaces between fibers.

1. Take 2 tubes of agar and a solution of Malachite green (365 g/mole) and a solution of Potassium permanganate (164 g/mole)
2. Mark the top of the agar on the outside of the tube (the starting point)
3. Add 10 drops of malachite green to one tube and 10 drops of Potassium permanganate to the other
4. Take note of the time
5. At 20 minute intervals, measure the distance from the top that the agar has moved. Do this for at least 1 hour.
6. Plot the data and compare the trends. Describe the rate of diffusion for each.



Hypothesize which solution will move faster through the agar and provide a reason.

Diffusion speed of dye molecules		
Dye	**Molecular Weight**	**Hypothesis** (fast or slow diffusion)
Malachite Green	365 g/mole	
Potassium Permanganate	164 g/mole	

Conclude:

- Which solution actually moved faster?
- Did this meet your expectations?
- Propose a reason why a certain dye moved faster.
- **Review through simulation**

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Activity: What makes my Gummy Bear swell faster?

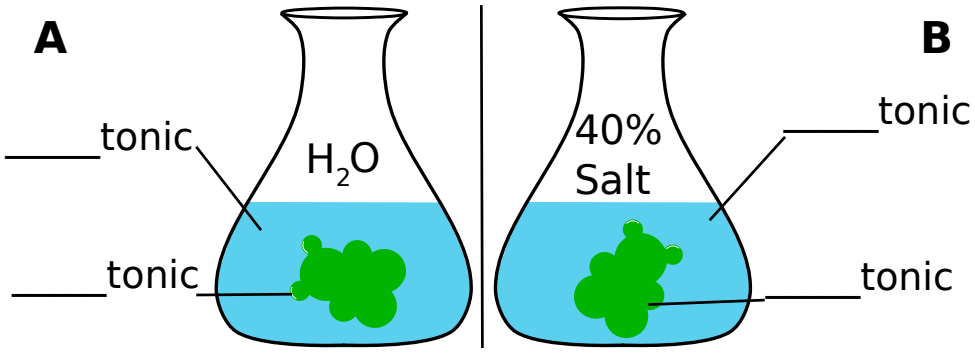
We're all familiar with gelatin (like the Jello brand). Gummy candies are are made of gelatin. Gelatin is a protein that exists as long fibers. When gelatin is dissolved in a liquid and cooled, the gelatin fibers tangle together in a mesh-like network. The space in between the gelatin molecules is filled with the fluid it was dissolved in. Gummy candies are considerably more firm than the gelatin molds we have as desserts because they contain a lot less fluid. Nonetheless, gummy candies are filled with a sugary solution with coloring. Like a cell, a gummy candy placed in solution will be affected by the properties of osmosis when submerged in different solutions.

Stop and think

- Is distilled water hypertonic, hypotonic or isotonic compared to the sugar solution inside a gummy candy?
- Based on your answer, hypothesize if a gummy candy submerged in distilled water or 40% salt solution will swell faster? Label the diagram below with your hypothesis.

Procedures

1. Obtain 2 gummy bears and place them in 2 different small flasks.
2. Drown 1 bear in distilled water and drown the other in 40% salt solution.
3. At the end of the lab session, remove the bears from solution and document the size difference with your mobile phone.



Condition	Tonicity Inside Bear Relative to the Solution	Tonicity Outside relative to the Bear	Hypothesis about swelling
A			
B			

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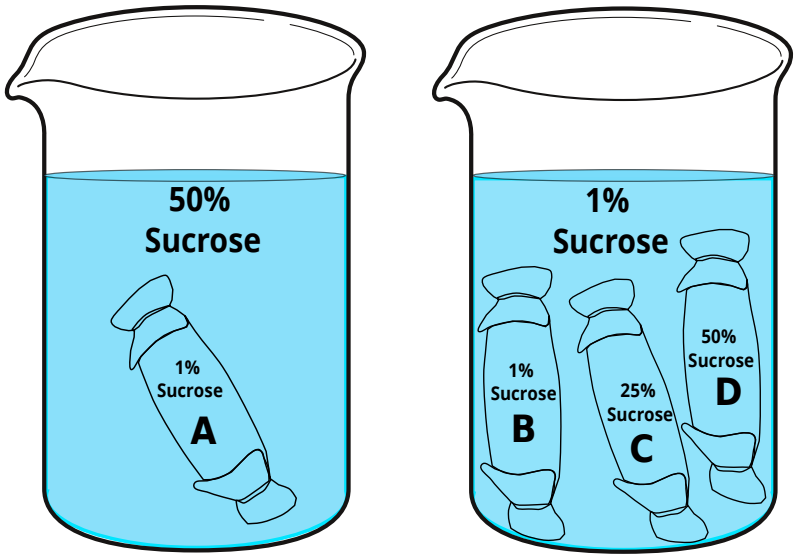
Observe Osmosis Along A Free Energy Gradient

1. Obtain four pieces of water-soaked dialysis tubing 15 cm long and eight pieces of string. Seal one end of each tube by tying it into a knot.
2. Open the other end of the tube by rolling it between your thumb and finger.
    1. write A, B, C, D on 4 pieces of paper
    2. insert the labels into individual bags
3. Fill the bags with the contents shown in the figure below with 10 ml of solution.
    1. **Bag A** 10ml 1% Sucrose
    2. **Bag B** 10 ml 1% Sucrose
    3. **Bag C** 10 ml 25% Sucrose
    4. **Bag D** 10 ml 50% Sucrose
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 gram.
6. Record the weights in **Data Table1: Weight of Dialysis Bags**.



7. Place bags B, C, and D in a beaker or large bowl filled with 1% sucrose. Record the time.
8. Place bag A in an empty beaker and fill the beaker with just enough 50% sucrose to cover the bag. Record the time.
9. Remove the bags from the beakers at 10-minute intervals for the next hour, blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers.
    - For each 10-minute interval record the total weight of each bag and its contents in **Data Table 1**.
    - Then calculate and record in **Data table 2: Change in Weight of Dialysis Bags** the change in weight from the initial weight.

Stop and Think:

- Define the tonicity of the solution inside the bag relative to the outside
- Based on your definitions, **hypothesize** the direction the solution will move (in or out of the bag) and fill in the table below

Hypothesized Movement of Solution based on Tonicity

Bag	Tonicity Inside Bag Relative to the Solution	Tonicity of Outside Solution relative to the Bag	Hypothesized solution movement (in, out, none)
A			
B			
C			
D			

Data Table 1: Weight of Dialysis Bags

Bag	0 Min	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min
A							
B							
C							
D							

Data Table 2: Net Weight Change of Dialysis Bags

Bag	0 Min (WT_0-WT_0)	10 Min (WT_{10}-WT_0)	20 Min (WT_{20}-WT_0)	30 Min (WT_{30}-WT_0)	40 Min (WT_{40}-WT_0)	50 Min (WT_{50}-WT_0)	60 Min (WT_{60}-WT_0)
A	**0**						
B	**0**						
C	**0**						
D	**0**						

Present your data:

- Plot your data using only the Change in Weight. (subtract the Initial Weight at 0 minutes from the Total Weight at each time point)

  Change in Weight = Total Weight$_{current_time}$ – Initial Weight$_{time_0}$
- Using a computer, create a scatterplot of the data from Table 2 and calculate the equation of the line

Conclude:

- Did your results match your hypotheses?
- What do the slopes of the lines generated from plotting Change Weight indicate to you?
- Can you analyze and articulate in words what has occurred with respect to these slopes?

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Why are cells so small?

1. Take 3 blocks of agar of different size (1cm, 2cm, 3cm) → these are our cell models
2. Measure the length, width and height of each cube using a ruler
3. Calculate the area of each face of the cubes and add all the areas together for a single cube
    - a cube has 6 faces → the total surface area is the same as the area of one side multiplied by 6
4. Calculate the volume of each cube
5. Report the surface area-to-volume in the table below

Data Table: Calculating Surface Area-to-Volume Ratio

Cell Model (cube)	Length	Width	Height	Total Surface Area	Volume of cell	Surface Area: Volume
1						
2						
3						

Stop and think:

- Which cube has the greatest surface area:volume ratio?
- Which cube has the smallest surface area:volume ratio?
- **Hypothesize**: In an osmosis or diffusion experiment, which cube size would have the greatest diffusion rate?

Procedures:

1. Each group will retrieve three agar cubes: A 3cm cube, a 2cm cube, and a 1cm cube.
2. Pour 200mL of 0.1M NaOH into your beaker.
3. Immerse your 3 cubes in the NaOH, noting the time.
4. Let the cubes soak for approximately 10 minutes.
5. Periodically, gently stir the solution, or turn the cubes over.
6. After 10 minutes, remove the diffusion solution
7. Blot the cubes with a paper towel.
8. Promptly cut each cube in half and measure the depth to which the pink color has penetrated. Sketch each block's cross-section.
9. Record the volume that has remained white in color.
10. Do the following calculations for each cube and complete the following data table:

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Data Table: Calculationof Diffusion Area-to-Volume

Cube Size	Cube volume (cm^3) V_{total}	Volume white (cm^3) V_{white}	Sketch of each Cube	Volume of the diffused cube ($V_{total} - V_{white}$) $V_{diffused}$	Percent Diffusion ($V_{diffused}/V_{total}$) % Diffused	Surface Area: Volume (from previous table)
1cm						
2cm						
3cm						

Conclude:

- Which cube had the greatest percentage of diffusion?
- Did this meet your expectations with your hypothesis?
- If you designed a large cell, would it be a large sphere or something long and flat?

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