DIFFUSION AND OSMOSIS

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I. OBJECTIVE:

To observe Brownian motion, diffusion, and osmosis.

II. BACKGROUND:

The cytoplasm of cells is 70 to 95% water. Dissolved or dispersed in that water are various salts, sugars, proteins, etc. which make up a complex mixture of molecules.

Molecules in liquids and gases are in constant motion due to their kinetic energy. Substances dissolve in water and disperse throughout a solution because they are constantly in motion. Diffusion is the tendency for molecules of any substance to spread out randomly into the available space. Substances will diffuse from more concentrated to less concentrated areas or solutions. Passive transport is diffusion across a biological membrane. Sometimes, though, solute molecules are too large to go through a semipermeable membrane. Osmosis (**osmo** = to push; **-sis** = the act of), then is a special case of passive transport in which water diffuses across a selectively permeable membrane from less to greater concentration to try to equalize the concentrations of the solutes. If a cell and its watery environment have the same concentrations of solutes, they are said to be isotonic (**iso** = equal; **tono** = tone, tension, stretched). If the environment has a greater concentration of solutes, it is hypertonic (**hyper** = over, above), and the cell will shrink as it loses water. If the environment has a lesser concentration of solutes, it is hypotonic (**hypo** = under, beneath) and the cell will swell or, in the case of animals, even burst as it gains water.

III. MATERIALS NEEDED:

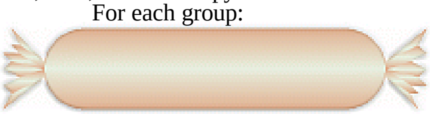
microscope, slide, coverslip
suspension of carmine particles
test tubes and rack
methylene blue (or another dye)
15% salt solution, dH_2O
Elodea
silver nitrate ($AgNO_3$) – caution: do not get on skin
dialysis tubing
balance
100 mL graduated cylinder(s)
25% solutions of salt (NaCl), glucose, sucrose, and albumin

IV. PROCEDURE:

OSMOSIS

The class should divide into groups of four or five people. Each group should test the following substances. All data gathered should be entered into the computer. You will, then, receive a copy of all the class data.

For each group:



A. Obtain five pieces of dialysis tubing approximately nine inches (20 cm) long. If not already cut and soaking, cut with scissors and soak each under cool, running tap water, rolling the end between your fingers until it opens up. Run tap water through it until it is completely open.

B. Tie a knot in one end of each tube to seal it, as close to the end as possible. Fill the tube with tap water, and while pinching the open end to hold it shut, gently squeeze the tube to check for leaks. If you find a leak, get a new piece of tubing.

C. For your group, obtain five 100 mL graduated cylinders and fill each approximately b to ¾ full with dH_2O. Label one for each of the solutions to be used.

D. Fill each tubing "bag" with one of the following solutions – each of the groups should test all five of these solutions.

1. tap water
2. 25% salt (NaCl) in dH_2O
3. 25% glucose ($C_6H_{12}O_6$) in dH_2O
4. 25% sucrose ($C_{12}H_{22}O_{11}$) in dH_2O
5. 25% egg albumin in saline solution

When filling the bags, leave them a little flaccid (**flacc** = flabby) or limp because later on they may absorb water and become turgid (**turg** = swell, swollen) or rigid, even to the point of bursting if too full.

E. Tie a knot in the open end of the "bag" to seal it. Avoid trapping air bubbles inside. When the tube is sealed, rinse it under tap water to remove any spills, then dry by gently rolling on a paper towel. Do not let it get overly dry – the surface of the bag should be dry enough to not mess up the balance, but should not start to dry out.

F. Using the electronic balance, weigh the bag to the nearest 0.01 gm and record the weight in your lab notebook and the computer. It will be quicker to record each weight in the computer as the bags are weighed rather than waiting to record all the weights at the end.

G. Gently submerge the bag into your cylinder of water.

H. Every 15 min. for the next 90 min., remove the bag and gently dry it as before, then weigh it. Record the weight in your lab notebook and in the computer. Note any change in the way the bag feels – does it increase or decrease noticeably in turgidity? Is there any visible evidence of the passage of molecules from the bag to the external water (color change)?

I. After you take your last reading, with the bag still out of the water, add a couple drops of silver nitrate ($AgNO_3$) to the water in the cylinder and observe what happens. If chloride ion (Cl^-) is present, it will react with the silver ions (Ag^+) to form silver chloride (AgCl) which is not water-soluble and, thus, shows up as a white precipitate by the following chemical reaction: $AgNO_3 + Cl^- \rightarrow AgCl\downarrow + NO_3^-$. Is there chloride ion present in your cylinder? **CAUTION: SILVER NITRATE STAINS SKIN BLACK UNTIL IT WEARS OFF – DO NOT GET THIS ON YOUR SKIN OR SPILL ANY!!!** Optionally, a flame test could be conducted to test for the presence of Na^+ and/or Benedict's Solution could be used to test for glucose in the cylinder water.

J. Do the following calculations for each time for each bag of solution:

1. For each time subtract the initial weight of the bag from the weight at the end of that time to determine the change in weight of that bag, or

$$\mathbf{wt_{fin} - wt_{ini} = \Delta wt}.$$

2. For each of these,

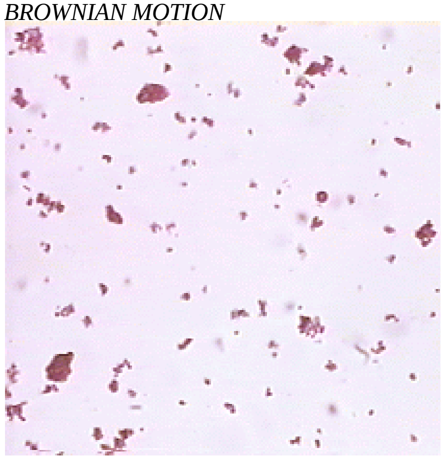
$$\mathbf{\Delta wt/wt_{ini} \times 100 = \%\Delta wt}$$

(percent change in weight).

3. In your notebook, make a graph of time (minutes from the start) on the X-axis, versus percent weight change on the Y-axis for each of your five bags (five lines).

K. Enter your data into the computer as you weigh each bag each time. While the bags are soaking, complete the rest of the lab exercise. Empty the tubes after testing.

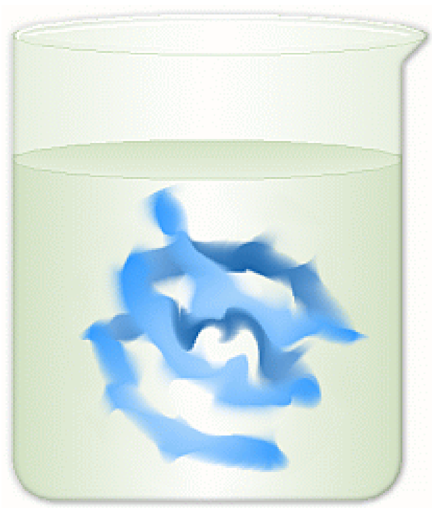
BROWNIAN MOTION

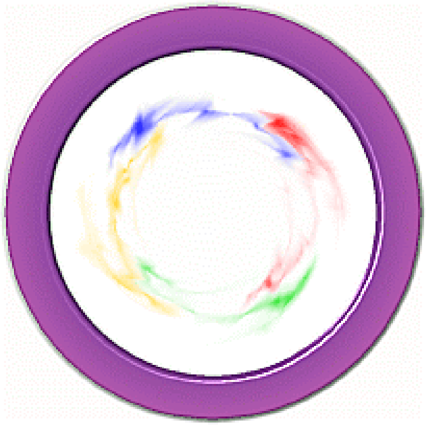


Make a wet mount of carmine particle (a red dye) suspension and examine under the microscope. Describe what you see. Are any of the particles, especially the smaller ones, moving? This type of motion is called Brownian motion after Robert Brown who first described it.

DIFFUSION

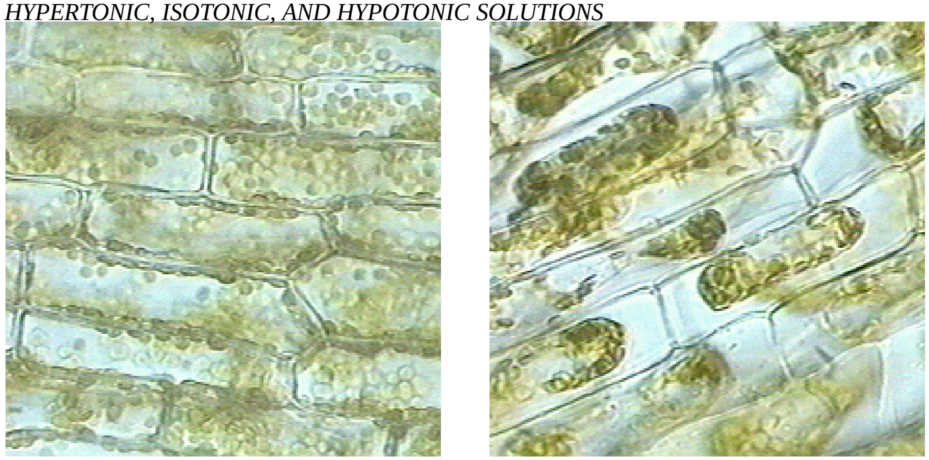
As a class, put some tap water in a beaker. Set the beaker on a table and let it sit until calm. Gently add a couple drops of methylene blue (or another dye) with the dropper near the surface to disturb the water as little as possible. Observe what happens over time. Do not bump or move the beaker once the dye is added.





Another similar demonstration that may be done if some milk is available, is to place some milk in a saucer. Gently add one drop each of red, yellow, green, and blue food coloring, each in a different quadrant of the milk. Then, gently add one or two drops of dish detergent to the center of the milk. Observe what happens.

HYPERTONIC, ISOTONIC, AND HYPOTONIC SOLUTIONS



(Plasmolysis in Plant Cells)

1. Make a wet mount of an *Elodea* leaf as before (using tap water). Examine and draw a typical cell as seen under the microscope.
2. Put a drop at a time of 15% salt solution at one edge of the coverslip and observe what happens to the leaf. DO NOT GET THIS ON THE MICROSCOPE!!! If some does get on the microscope, wipe it off immediately and thoroughly! Record how many drops of salt solution were added to cause a change in the cells. Draw and describe this change.
3. Now, add distilled water a drop at a time. DO NOT GET THIS ON THE MICROSCOPE!!! If some does get on the microscope, wipe it off immediately and thoroughly! If the slide gets too wet, absorb some of the water with a paper towel or Kimwipe. Observe what happens and record how many drops of water were needed to cause a change in the cells. Draw and describe this change.

WHEN YOU ARE DONE: make sure your microscope is CLEAN AND DRY before putting it away. Make sure there is no salt solution spilled on it – especially check around the hole where the condenser comes up through the stage. Remember to follow all the steps for proper storage of the microscope (4× lens down, rheostat down, light off, cord coiled neatly, stage down, cover on).

V. DATA:

Record all observations, draw pictures wherever possible, indicate colors of objects observed, and label all recognizable cell organelles. For "Diffusion" a chart of time and observations may be helpful. Enter "Osmosis" data into the computer.

VI. DISCUSSION:

1. What does the presence of chloride ion in the water in the cylinders in "Osmosis" indicate was happening – where did the chloride ion come from?

2. What do you think caused the Brownian motion you observed? Could something (what?) have been bombarding and jostling the particles to cause them to move? From this, can you figure out why you hear sound even in a quiet room?

3. In "Diffusion," does the <u>rate</u> of diffusion just after adding methylene blue change by 15 to 30 minutes later?

4. In "Hypotonic/Hypertonic Solutions," when you first examined the *Elodea* leaf, could you see the plasma membrane? Explain what caused the change when you added saltwater. What happened to the plasma membrane when the saltwater was added? What is responsible for keeping the membrane in its normal position? What happened to the plasma membrane when distilled water was added? Do you think it would be possible that this cell could explode? Why/why not?

5. Plant cells have cell walls and animal cells do not. Predict what you might expect to see if you added 15% salt to a solution of blood cells. What if you added distilled water to them?

6. If the milk-food coloring demonstration was done, this is actually a rather complicated system. Milk is an emulsion, and detergent is an emulsifying agent. However, detergent is also a **surfactant**, a substance which reduces the surface tension of water and/or similarly affects the tension at the boundary of water with another liquid. Chemicals like food coloring tend to be "big" organic molecules of some sort. Based on your knowledge of biology and chemistry, suggest possible explanations for what might be going on here.