Lab 2- Molecular Activity and Membrane transport

Purpose

These experiments helped observe and investigate the certain aspects of passive transport such as diffusion, osmosis and differential permeability and the concepts of filtration and the effects of tonicity of cells.

Procedures

2-B: Measurement of diffusion through a liquid

1. Working in groups, fill three Petri dishes with 40 ml. of 25C water.
2. Drop one crystal of potassium permanganate into each dish. Be sure to use the same amount of potassium permanganate for each dish. Record the time.
3. Measure,in millimeters, and record the largest diameter of the colored spot after 5 minutes.
4. Repeat steps 1-3 for water at 5C and at 45 C.
5. Construct a graph of ranges and means for each temperature.
6. Based on your knowledge of diffusion, what is an explanation for these results?

2-C: Measurement of diffusion through agar

1.Petri dishes have been filled with agar. Two holes have been made in the agar. Into one hole, place two drops of methylene blue. Into the other hole, place two drops of potassium permanganate.Record the time and immediate diameter of each spot. This Will be your time zero measurement.
2.Measure the diameter of each spot in millimeters once every minute for fifteen minutes.Calculate the averages from the data collected by all groups doing this exercise.Summarize these data.
3.Construct a graph of average diffusion diameter versus time for both chemicals.

4.Determine the diffusion rate of each chemical. Which has the fastest diffusion rate,methylene blue or potassium permanganate? Record these results.
5.Look up the molecular formula and structure of methylene blue and potassium permanganate in a Merck Index. Make note of this information.
6.Interpret your result with respect to the information obtained from the Merck Index.

2-D: Demonstration of filtration

1. Fold three filter papers into cones and insert them into three separate glass funnels. Wetthe papers to make them stick to the glass.
2. Prepare three 100-milliliter solutions of charcoal and water. Make one thick, one medium thickness, and one thin. Record the mass of the charcoal used in each preparation.NOTE: if your “thin” solution continually runs through the filter, making it impossible to count drops, it is too thin; you will need to make all your solutions proportionally thicker.
3. Pour 50 ml of each solution, one at a time, into a funnel.
4. Immediately count the number of drops produced per minute.NOTE:it may be easier to count the drops for 15 seconds then multiply by four to obtain drops per minute.
5. Count the number of drops per minute when the funnel is half-filled.
6. Count the number of drops per minute when the funnel is nearly empty.
7. Did the charcoal pass into the filtrate? Which solution had the fastest rate of filtration?What is the driving force behind filtration? Whatother factors influence the rate of filtration? Do your results illustrate these influencing factors?
8. Repeat these procedures with the remaining 50 ml. of solution.

2-F: Measurement of osmosis

1. Attach dialysis bags filled as much as possible with sucrose solutions securely to the bottom of two open, thin glass tubes. One bag should be filled with a 25% sucrose solution and the other should be filled with a 50% sucrose solution. Make sure ends of the tubes are immersed in the

solutions.NOTE: reliable results depend on your ability to tightly seal the dialysis bags.
2. Insert both bags into separate beakers of distilled water making sure the dialysis bags are fully submerged but not touching the bottom of the beakers, and suspend each by gently applying a ring stand clamp to the glass tubes. Check for solutions leaking out of the bags.
3. Allow five minutes for the systems to equilibrate.Then, mark the fluid levels of each glass tube with a felt pen.Record the time.
4. Record the fluid level of the glass tubes in millimeters every 10 minutes for 50 minutes.
5.If the fluid level rises to the top of the glass tube sooner than 50 minutes, record the time it took to get there, measure the length in millimeters from the equilibration line to the top of the glass tube. Divide that length by the number of minutes to get your rate in mm/min.
6.Determine the rate of osmosis for each system. Which system had the fastest osmotic rate, the 25% or 50% sucrose solution? Explain these results.

2-G: Measurement of differential permeability of sugar and starch NOTE: In this experiment, chemical indicators will be used to determine the presence of starch and sugar. Lugol's solution, an amber iodine-containing reagent, will turn dark navy blue in the presence of starch. Benedict's solution, a blue cupric (Cu+2)solution, when heated in the presence of a reducing sugar, will be reduced to form a reddish precipitate of cuprous oxide (Cu2O). Benedict's solution will change different colors, ranging from green to red, depending upon the amount of sugar present.

1. Fill a dialysis bag with a 1% starch–10% glucose solution. Reliable results depend on your ability to tightly seal the dialysis bag.
2. Tie the bag to a glass rod and suspend it in a beaker of distilled water.NOTE: Test the water from the bottom of the beaker to ensure that it is free of starch and/or sugar.
3. After 15 minutes has passed, check the water again for starch and sugar in the following way:Test for starch:a. Add 10 drops of Lugol's solution to 5

ml of water obtained from the beaker.Reddish color = No starchNavy blue color = Starch present
Test for sugar:A. Add 3 ml of Benedict's solution to 5 ml of water obtained from the beaker.Simmer the solution at a low boil for 5 minutes.Blue color = No sugar Color change = Sugar present (green = little sugar; yellow =moderate sugar;orange = more sugar; red = lots of sugar)
4. Test the water in the beaker again at 30, 45 and 60 minutes.5. Record these results. Explain the significance of these findings in relation to the permeability of the dialysis bag.
5. Record these results. Explain the significance of these findings in relation to the permeability of the dialysis bag.

2-H: The effects of tonicity on red blood cells-Demonstration Tonicity refers to the solute concentration of solutions.Hypertonic Solutions have a higher solute concentration than the cells in this solution. Red blood cells should shrink or create due to osmotic loss of water in hypertonic solutions.Hypotonic Solutions have a lower solute concentration than cells in this solution. Red blood cells in a hypotonic solution swell and will eventually undergo cytolysis due to osmotic gain of water.Isotonic Solutions have the same solute concentration as cells in this solution. Red blood cells in isotonic solutions slightly swell and shrink in a dynamic equilibrium with their medium. In This exercise, the effect of hypertonic, isotonic, and hypotonic solutions on red blood cells will be observed.

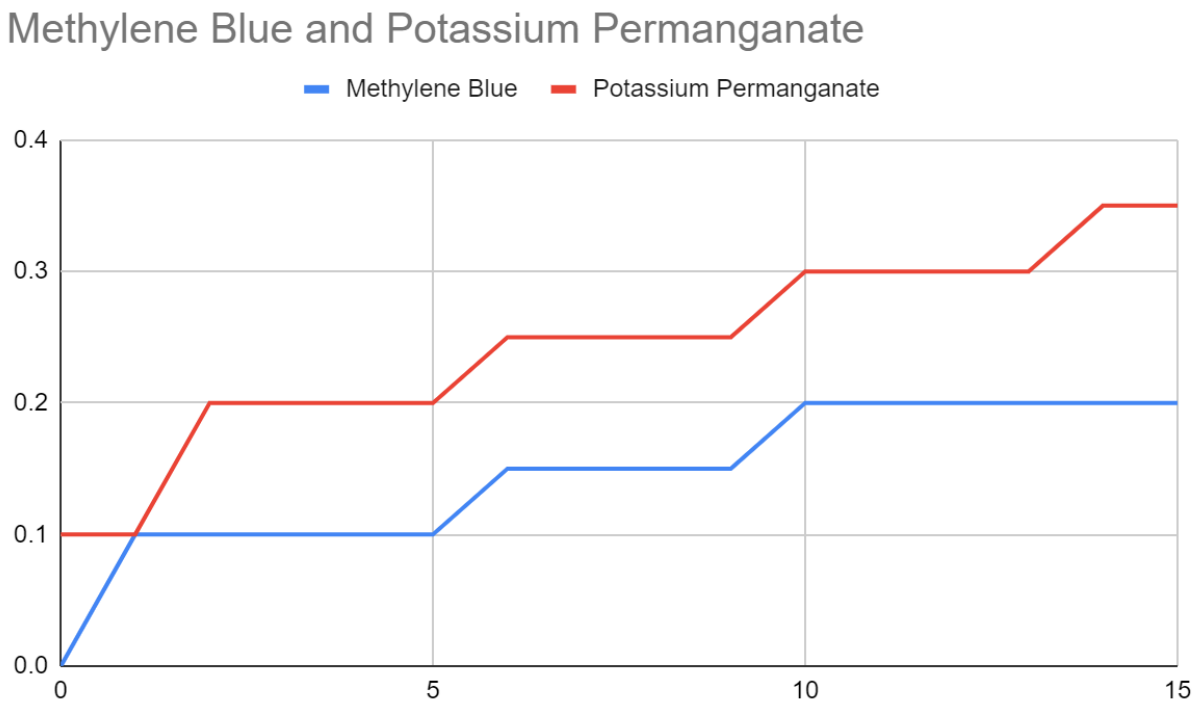
1. One milliliter of each of the following solutions will be in three separate test tubes.a. Distilled water (hypotonic)b. Physiological saline–0.85% NaCl (isotonic)c. Salt water–2.0% NaCl (hypertonic)
2. A small drop of blood will be added to each tube and the contents thoroughly mixed.
3. A wet mount slide will be made of each solution.
4. Examine each slide under the high-dry lens of a compound microscope.
5. Observe the following:a. Hemolysis of cells in the hypotonic solution. (Note the transparent solution.)b. Maintenance of cell size in the isotonic solution.c. Crenation of cells in the hypertonic solution.
6. Make a drawing of each observation and provide an explanation for each

Results

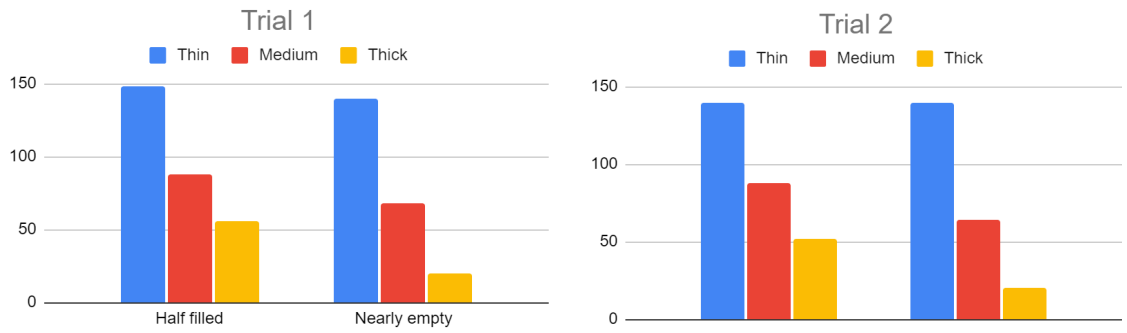
2-B

Time	Hot	Room temp	Cold
5	5.5mm	5mm	4mm

2-C



2-D



2-F

	fifty	twentyfive
0	42.71g	54.94g
10	46.23g	49.25g
20	52.32g	47.56g
30	52.84g	46.03g
40	52.93g	45.74g
50	53.17g	45.23g

2-G

Time	A	B
15	no starch	little sugar
30	no starch	moderate sugar
45	no starch	more to lot of sugar
60	no starch	a lot of sugar present

Discussion

In this laboratory, there were some different experiments that were done and observed that helped introduce different concepts. In the experiment measurement of diffusion through agar, it appeared that the potassium permanganate had a faster rate of diffusion than the methylene blue and it felt like that was the case since potassium permanganate has a smaller size, high solubility, ionic nature, and a steeper concentration gradient. In the demonstration of filtration experiment, the thickest solution mixed with charcoal and water was the substance that had the least counted amount of drops per minute that were pouring out of the funnel while the thin solution had the most counted amount of drops per minute that were pouring out of the funnel. I observed that the charcoal did pass into the filtrate along with the thin solution being the fastest rate of filtration. Hydrostatic pressure is the main driving force behind filtration along with

the factors of the size and shape of particles, concentration of particles, viscosity of the fluid and temperature. Based on the results from the experiment, it looked like the viscosity of the fluid and maybe the size, shape and concentration of particles were some of the influencing factors. In the effects of tonicity on red blood cells experiment, I observed all three of the solutions by using a microscope. What I noticed is that in the distilled water solution, there is not a lot there except a black line on the right and some kind of blue line on the left, in the physiological saline solution there seems to be some cells that form with one area on the bottom right that the cells are really noticeable while having the same black line as the distilled water and in the saltwater solution, you can see all the cells that you never got to see from the physiological solution along with that black line. I noticed that all of the solutions have a black line to it and that the more percentage that a certain solution has, the more cells that you start to see that's behind the black line.

Conclusion

In conclusion, these experiments are a part of the certain aspects of passive transport such as diffusion, osmosis and differential permeability along with the concepts of filtration and the effects of tonicity of cells. This lab allowed others to do things like understanding the mechanism of Brownian motion, the difference between passive and active transport, being able to define: diffusion, osmosis, active transport, dialysis,and filtration, to know the result of dropping red blood cells in hypertonic, isotonic, and hypotonic solutions and understanding the significance of all of these experiments in terms of passive transport processes and molecular activity.