Biology 125- Human Physiology

Laboratory 2- Molecular Activity and Membrane Transport

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I. Purpose

The purpose of this lab seeks to demonstrate how both passive and active transport systems are used to transfer materials into and out of cells. These experiments assist in demonstrating the characteristics of semi-permeability, osmosis, and diffusion as they relate to the movement of several materials through various membranes.

II. Procedure

2-B: Measurement of diffusion through a liquid

1. Fill three Petri dishes with 40 ml. of 25°C water.
2. Drop one crystal of potassium permanganate into 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 5°C and 45°C.
5. Construct a graph of ranges and means for each temperature.

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 the data.
3. Construct a graph of average diffusion diameter versus time for both chemicals.
4. Determine the diffusion rate for 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 Merck Index. Make note of this information.

6. Interpret your result with respect to the information obtained from Merck Index.

2-D: Demonstration of filtration

1. Fold three filter papers into cones and insert them into three separate glass funnels. Wet the papers to make them stick to the glass.
2. Prepare three 100-millileter solutions of charcoal and water. Make one thick, one medium thickness, and one thin. Record the mass of 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? What other 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 filled with 50% sucrose solution. Make sure ends of the tubes are immersed in the solution
   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 solution 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 the 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). The 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 starch
      Navy blue color = Starch present

   Test for starch:
   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.

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 crenate 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 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.

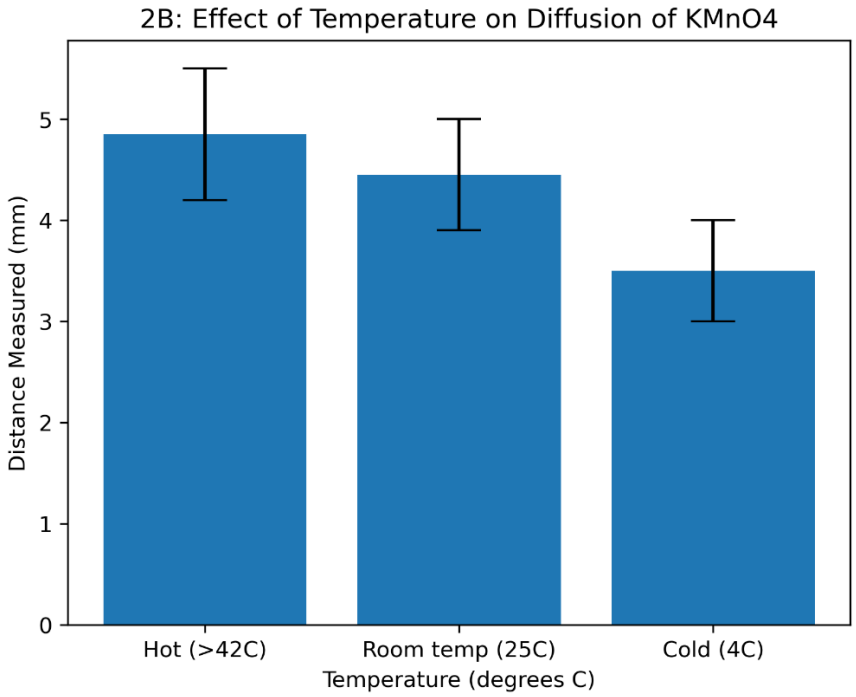
1. One milliliter of each of the following solutions will be in three separate test tubes.
    a. Distilled water (hypotonic)
    b. Physiological saline – 0.85% NaCl (isotonic)
    c. Salt water – 2.0% NaCl (hypertonic)
2. A small drop of blood will be added to each tube and the contents thoroughly 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.

Molecular formula for methylene blue C16H18ClN3S molecular weight 319.85
Molecular formula for potassium permanganate KMnO4 Molecular weight 158.03

III. Result

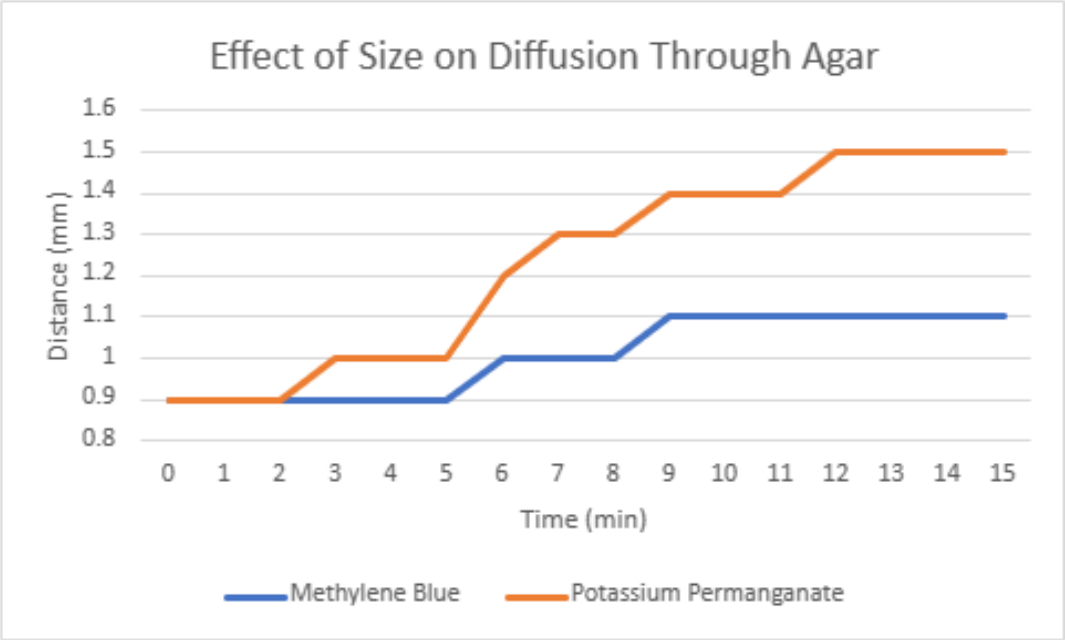
2-B: Measurement of diffusion through a liquid

Hot (>42°C)	Room Temperature (25°C)	Cold (4°C)
4.85mm	4.45mm	3.5mm



2-C: Measurement of diffusion through agar

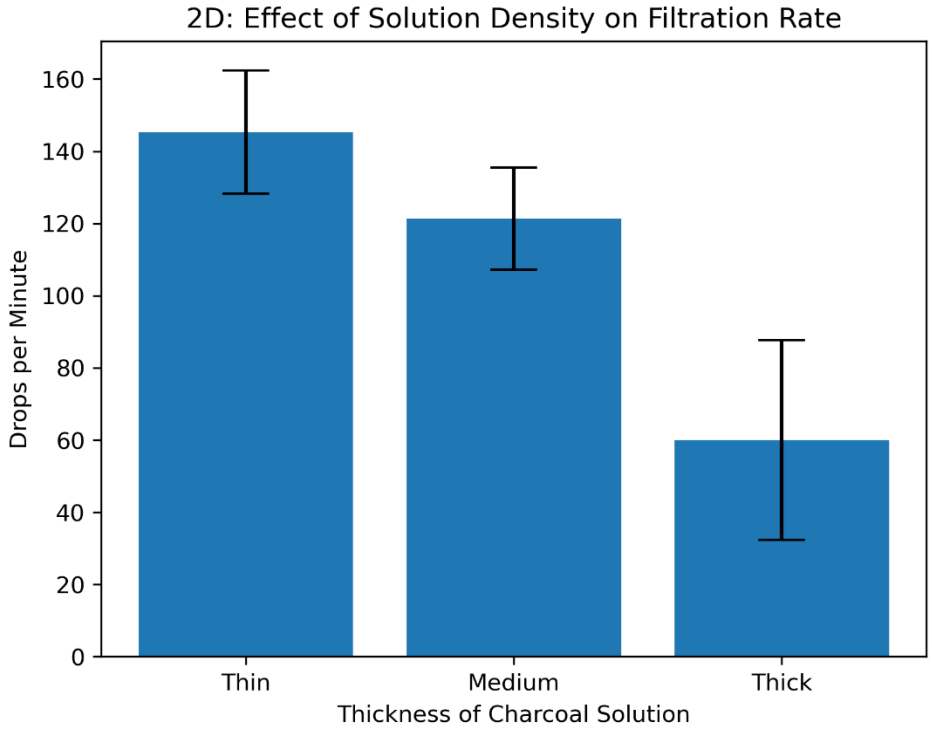
Time	Methylene Blue	Potassium Permanganate
0	0.9mm	0.9mm
1	0.9mm	0.9mm
2	0.9mm	0.9mm
3	0.9mm	1mm
4	0.9mm	1mm
5	0.9mm	1mm
6	1mm	1.2mm
7	1mm	1.3mm
8	1mm	1.3mm
9	1.1mm	1.4mm
10	1.1mm	1.4mm
11	1.1mm	1.4mm
12	1.1mm	1.5mm
13	1.1mm	1.5mm
14	1.1mm	1.5mm
15	1.1mm	1.5mm



2-D: Demonstration of filtration

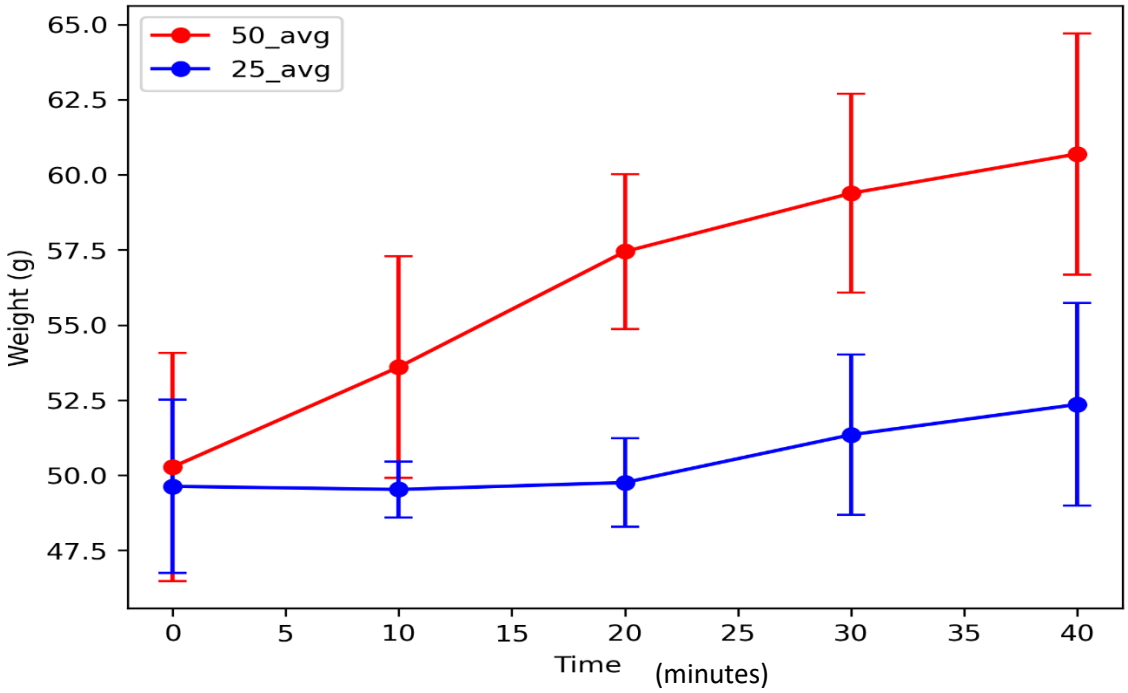
Data:

Thin	Medium	Thick
168, 112, 156	148, 100, 116	60, 12, 108
Mean: 145.33	Mean: 121.33	Mean: 60



2-F: Measurement of osmosis

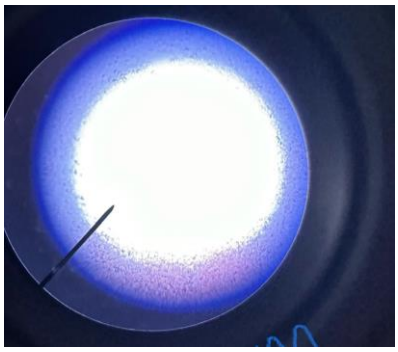
Time	Group 1 (50)	Group 1 (25)	Group 2 (50)	Group 2 (25)	Group 3 (50)	Group 3 (25)	50 Average	50 Average	25 Average	25 Average
0	42.71	54.94	54.59	48.95	53.53	45.00	50.276667	49.630000	3.795688	2.889504
10	46.23	49.25	57.31	51.26	57.26	48.07	53.600000	49.526667	3.685028	0.931206
20	52.32	47.56	59.57	52.55	60.44	49.16	57.443333	49.756667	2.573949	1.471058
30	52.84	46.03	61.76	53.75	63.55	54.26	59.383333	51.346667	3.312221	2.662407
40	52.93	45.74	62.81	54.52	66.34	56.81	60.693333	52.356667	4.013196	3.373734
50	53.17	45.23	NaN	NaN	68.64	57.87	60.905000	51.550000	6.315601	5.160258



2-G: Measurement of differential permeability of sugar and starch

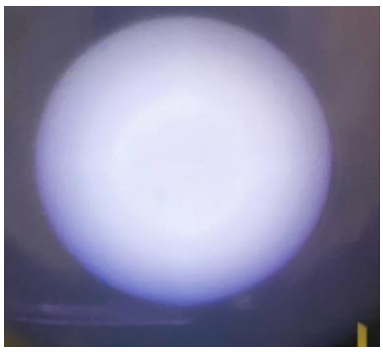
Time	Starch	Sugar
15 minutes	No starch present	Little sugar present
30 minutes	No starch present	Moderate sugar present
45 minutes	No starch present	More to lots of sugar present
60 minutes	No starch present	Lots of sugar present

2-H: The effects of tonicity on red blood cells – Demonstration



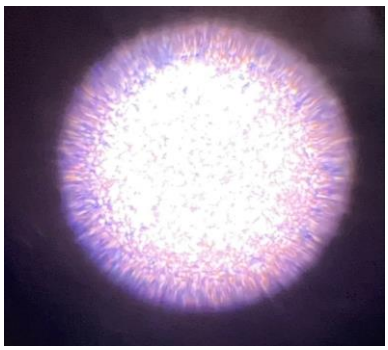
Physiological saline

0.85% NaCl (isotonic)



Distilled Water

(hypotonic)



Salt Water

2% NaCl (hypertonic)

IV. Discussion

Six experiments were completed and by far my favorite one was seeing the diffusion of starch and sugar through a dialysis bag. It is interesting to learn about particle size and diffusion of different elements.

V. Conclusion

I learned today that diffusion during the dialysis bag experiment and diffusion through agar are solely dependent on temperature and size. Filtration is dependent upon the solutions density, as proven in experiment, 2-D: Demonstration of filtration. Overall this lab helped me understand the significance of each experiment in terms of passive transport processes and molecular activity.