



**Salt Imbalance and Osmosis -
Thick Mucus in Cystic Fibrosis
Teacher instructions and answers**

Introduction - In this exercise you will explore how the salt imbalance resulting from a dysfunctional chloride channel could, through osmosis, lead to the thick mucus characteristic of cystic fibrosis. This exercise will demonstrate one of the possible mechanisms by which the cystic fibrosis mutation could lead to disease. Other possible mechanisms will be discussed following the exercise.

Background knowledge required!!

This exercise assumes that students are already familiar with cell membrane structure, ion channels, and osmosis. A handout "Brief Review of Membrane Structure and Osmosis" is provided to help refresh students' memories.

Teacher Prep Materials

Supplies to purchase

Dialysis tubing - Carolina (800)334-5551

AA-68-4212 - 1 inch by 10 ft (enough for 10 groups) \$3.60

AA-68-4214 - 1 inch by 50 ft (enough for 50 groups) \$15.95

AA-68-4216 - 1 inch by 100 ft (enough for 100 groups) \$30.50

Dialysis tubing can be stored dry in the refrigerator for a long time.

It needs to be cut into 6 inch pieces and soaked in water prior to use.

It will mold if left for extended periods of time (months) in water.

Other materials (per group)

2 500 ml beakers

1L 0.9% NaCl (9 g/L)

100 mls 20% NaCl (200 g/L)

balance (one per class is sufficient, more could be helpful)

paper towels

droppers to transfer liquid into the dialysis tubing

2 pieces of dialysis tubing soaked in water

Answers

Predictions

Think about what you know about membrane structure, what substances freely cross membranes, and in which direction they can move freely. If necessary review the information in the box on membranes. Then answer the questions on the following page. *Anticipated predictions are noted below. Note though that these are just predictions, so other answers are acceptable.*

1. If the Non-CF "cell" were placed in a beaker of 0.9% NaCl, is the concentration of NaCl higher on the inside or the outside of the cell, or equal?

Salt concentration would be the same outside as inside the cell

Would chloride enter the cell, leave the cell, or stay where it is?

Chloride ions can not cross the membrane and would remain where they are

Would water enter the cell, leave the cell, or stay where it is?

There would be no net movement of water in either direction.

2. If the cystic fibrosis "cell" were placed in a beaker of 0.9% NaCl. Is the salt concentration higher on the inside or the outside of the cell or equal?

Salt concentration would be higher inside the cell than outside the cell

Would chloride enter the cell, leave the cell, or stay where it is?

Chloride ions are unable to cross the cell membrane, and since CF cells have no chloride ion channels, chloride ions would stay inside the cell

Would water enter the cell, leave the cell, or stay where it is?

Water would enter the cell to dilute out the high chloride concentration there

Procedure

1. Get two beakers, label one cystic fibrosis and the other normal. Put 400 mls 0.9% NaCl into each beaker.
2. Obtain a piece of dialysis tubing. It is important when to work carefully with dialysis tubing as it will get very small holes very easily. Tie a knot in one end. To make the knots easier to tie, twist the end about 1.5 inches and then tie this twisted portion. Fill the dialysis tubing with 0.9% NaCl (the concentration of NaCl usually found in the body). You want to add an amount of NaCl so that when tied, the bag will be full to about half capacity without a large air bubble. This will allow substances to diffuse in both directions across the membrane. When the bag is tied shut on the other end, you should be able to squeeze the bag in the middle and have the two opposite walls touch.
3. Blot any excess liquid from the outside of the bag. Weigh this cell. Note the weight in the table on the following page.
4. Put this cell into the NaCl solution in the bottom of the "normal" beaker. Every 5 min for the next 20 min, remove the bag from the beaker, blot it gently, weigh it, and then return the bag to the beaker. Note your weights in the table. While waiting, set up the second bag as described below.
5. Obtain a second piece of dialysis tubing. Tie a knot in one end. Fill the dialysis tubing with 20% NaCl following the same procedure used above.
6. Blot any excess liquid from the outside of the bag. Weigh this cell. Note the weight in the table on the following page.
7. Put this cell into the NaCl solution in the bottom of the "cystic fibrosis" beaker. Every 5 min for the next 20 min, remove the bag from the beaker, blot it gently, weigh it, and then return the bag to the beaker. Note your weights in the table.

Be sure bags are not overfilled. If they are overfilled, water will be unable to enter the bag.

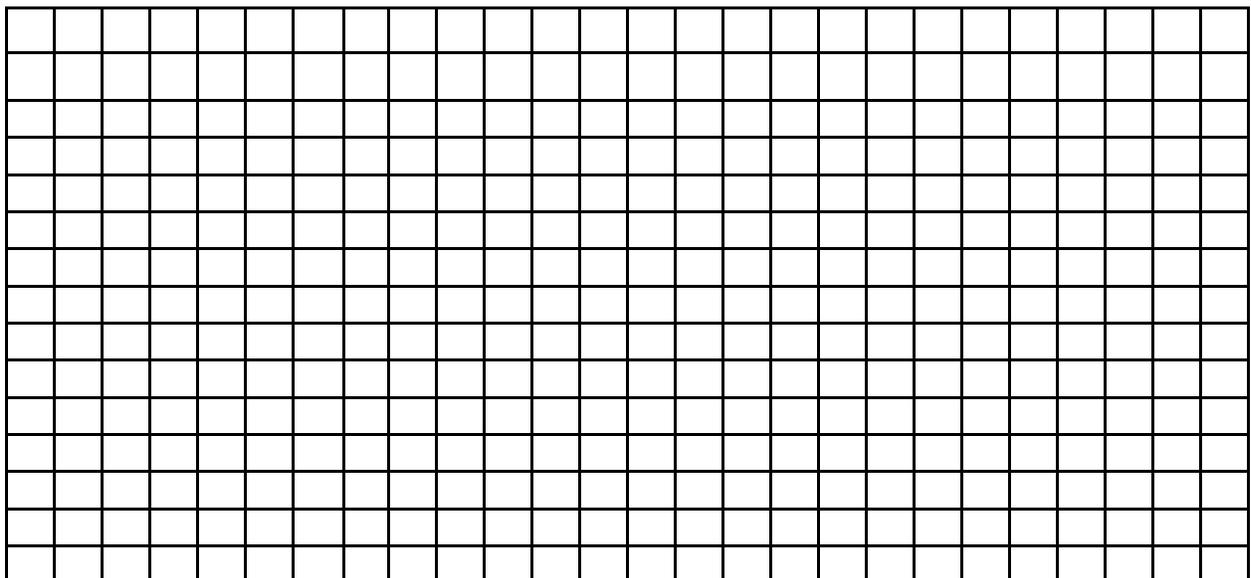
Note that in reality NaCl will cross a dialysis membrane (although it will not cross a cell membrane). However, the experiment will still work.

Results -

	Contents of bag			
	0.9% NaCl		20% NaCl	
Time	Weight of bag (g)	Weight of bag minus starting weight (g)	Weight of bag (g)	Weight of bag minus starting weight (g)
Start (0 minutes)		0		0
5 minutes		<i>Weight should remain the same over time</i>		<i>Weight should increase over time</i>
10 minutes				
15 minutes				
20 minutes				

Graph the results

On the graph paper below plot the change in weight over time, with time on the x-axis and change in weight on the y-axis. Draw two different lines, one for the 0.9% NaCl, one for the 20% NaCl. Be sure to label the axes, including units.



Analyze the results.

Did water move into or out of the bag? What does the difference in weight of your bag indicate about the movement of water?

A decrease in the weight of the bag would indicate movement of water out of the bag.

The weight of the bag remaining the same would indicate no net movement of water

This is what should have happened with the bag containing 0.9% NaCl.

An increase in the weight of the bag indicates movement of water into the bag.

This is what should have happened with the bag containing 20% NaCl.

Student results may vary depending on how full they fill the dialysis bags. It is important that the dialysis bags not be filled too full.

How do your results compare with your predictions?

Will vary depending on student results and predictions. Just be sure the student has correctly decided whether their results are the same, or contradict, their predictions.

If you are unable to make a firm conclusion, discuss how you would change your experiment.

Based on your results, why might the mucus of CF patients be thicker than that of non-CF individuals?

Due to the high concentration of chloride ions in the CF cells, water will leave the mucus and move into the epithelial cells lining the airway. This will result in dehydrated, thick mucus.

Follow up information - Alternative Explanations

Scientists actually do not understand exactly how this chloride channel defect causes the symptoms of cystic fibrosis.

In this experiment, you explored one of the two main possible explanations that scientists are pursuing at this time (others may appear later). In the explanation that you explored, chloride is unable to leave epithelial cells and as a result, the chloride ion concentration is higher within epithelial cells which then take up water resulting in thick, dehydrated mucus. This theory is supported by the fact that patients have very thick mucus, both in the lungs, and in the digestive tract. Further, some in vitro experiments have shown increased osmosis of water into cells with a CF mutation.

The second alternative that scientists are pursuing is that NaCl concentrations are actually higher outside the respiratory epithelial cells. This high NaCl concentration may inhibit the action of antibiotics (defensins) that our respiratory tract produces, thus resulting in repeated respiratory tract infections. Evidence to support this theory includes the isolation of defensins, and showing that they are sensitive to high levels of NaCl. Also, in vitro experiments different from those described above have documented increased NaCl concentrations outside cells with a CF mutation.

Note that the two alternative explanations are contradictory to one another; one suggests higher chloride ion concentrations within CF cells, the other suggests higher chloride concentrations outside the CF cells. A major problem in addressing this question is the inability at this time to measure the NaCl concentration of the epithelial surface in a patient. Salt concentrations in expelled mucus can be measured, but vary greatly, and may not reflect the salt concentration of liquid still in a human in direct contact with the epithelial cell layer.

Further research should help sort out these conflicting results and lead to a better understanding of the molecular mechanisms underlying cystic fibrosis.