

Molecular biology paper

[Science](#), [Biology](#)



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Introduction

A cell's plasma membrane is known to be selectively permeable. This implies that the membrane is selective on what substances can pass in and out of the cell. There are two methods of transport that occur through the plasma membrane. One method of transport is called the active process which uses ATP energy to transport substances through the membrane. The other method is called a passive process which does not require the use of ATP energy. During passive processes, molecules are transported through the membrane by differences in concentration or pressure between the inside and outside of the cell.

Two important types of passive processes are diffusion and filtration. Every cell in the human body uses diffusion as an important transport process through its selectively permeable membrane. During diffusion, molecules that are small enough to pass through a membrane's pores or molecules that can dissolve in the lipid section of a membrane move from an area of higher concentration to an area of lower concentration. The kinetic energy

that all molecules possess is the motivating force in diffusion. Facilitated diffusion occurs when molecules are too large to pass through a membrane or are lipid insoluble.

In this process, carrier protein molecules located in the membrane combine with solutes and transport them down the concentration gradient. Filtration is another type of passive process and, unlike diffusion; this is not a selective process. The pressure gradient on each side of the membrane as well as the membrane pore size depends on the number of solutes and fluids in the filtrate. During filtration, water and solute molecules pass through a membrane from an area of higher hydrostatic pressure to an area of lower hydrostatic pressure.

This means that water and solutes would pass through a selectively permeable membrane along the pressure gradient. To gain a better understanding of a cell's selectively permeable membrane and the passive processes of simple diffusion facilitated diffusion, and filtration, three experiments were conducted.

Materials and Methods

Activity 1: Simulating Dialysis (Simple Diffusion)

Materials:

- two glass beakers
- four dialysis membranes: 20 (MWCO), 50 (MWCO), 100 (MWCO), and 200 (MWCO)
- membrane holder

- membrane barrier
- four solutes: NaCl, Urea, Albumin, and Glucose solution dispenser
- deionized water
- timer
- beaker flush

This experiment was conducted first by placing the 20 (MWCO) dialysis membrane into the membrane holder.

The membrane holder joined the two glass beakers; one on the left side and one on the right side. Then, 9.00 mM of NaCl concentration was dispensed into the left beaker. Deionized water was dispensed in the right beaker. When the timer was started, the barrier that surrounded the membrane holder was lowered to allow the contents of each beaker to come in contact with the membrane.

After the 60 minutes of compressed time elapsed, results were read and recorded. Finally, each beaker was then flushed for preparation of the next experiment run. These exact steps were followed using each dialysis membrane size (20, 50, 100, and 200) as well as with each solute (NaCl, Urea, Albumin, and Glucose). There were a total of sixteen runs in this experiment.

Activity 2: Simulating Facilitated Diffusion

Materials:

- two glass beakers
- membrane builder

- membrane holder
- glucose concentration
- solution dispenser
- deionized water
- timer beaker flush

In this experiment, the first step was to adjust the glucose carrier to 500 in order to correctly build the membrane. Next, a membrane was built in the membrane builder by inserting 500 glucose carrier proteins into it. Then, the newly built membrane was placed into the membrane holder that joined the two glass beakers. The two glass beakers were joined on the left and right sides of the membrane holder. After that, 2.00 mM of glucose concentration was dispensed into the left beaker. The right beaker was filled with deionized water.

The barrier around the membrane holder dropped when the timer was started. After 60 minutes of compressed time elapsed, the results were read and recorded. Finally, both glass beakers were flushed to prepare for the next experimental runs. The above-mentioned steps were repeated by increasing the glucose concentration to 8.00. Both the 2.00 mM and the 8.00 mM glucose concentration solution was tested using membranes built with 500, 700, and 900 glucose carrier proteins. There were a total of six experimental runs.

Activity 4: Simulating Filtration

Materials:

- two glass beakers membrane holder
- 4 dialysis membranes: 20 (MWCO), 50 (MWCO), 100 (MWCO), and 200 (MWCO)
- 4 solutions: Na+Cl₂, Urea, glucose, and powdered charcoal
- solution dispenser
- pressure unit
- timer
- filtration rate indicator
- membrane residue analysis analyzer
- beaker flush

In the final experiment, the two glass beakers were placed one on top of the other with the membrane holder between them. The pressure unit that rested on the top beaker was used for forcing the solution from the top beaker through the selected membrane and into the bottom beaker.

The bottom beaker contained nothing; however, the filtration rate indicator was attached to it from one side. The experiment began by placing the 20 (MWCO) dialysis membrane into the membrane holder. Then, 5.00 mg/ml of each of the following solutions: Na+Cl₂, Urea, glucose, and powdered charcoal was dispensed into the top beaker. The pressure unit was adjusted to 50 mmHg of pressure. The timer was set to 60 minutes of compressed time and when the timer started, the membrane holder retracted. The solution then flowed through the membrane and into the beaker underneath.

When the timer stopped, the membrane was then placed in the membrane residue analysis analyzer. The results were read and recorded and the

beakers were flushed for the next experimental runs. All the above steps were repeated using the 50 (MWCO), 100 (MWCO), and 200 (MWCO) membranes.

Results

Table 1: Activity 1: Simulating Dialysis (Simple Diffusion)

Key: Solutes that we're able to diffuse into the right beaker are indicated by a "+". Solutes that were not able to diffuse into the right beaker are indicated by a "-".

Membrane (MWCO) Solute (9.0 mM)	(Pore Size)	NaCl	Urea	Albumin	Glucose
	20	-	-	-	-
	50	+	-	-	-
	100	+	-	-	-
	200	+	-	-	+

Graph 1: Activity 2: Simulating Facilitated Diffusion Glucose Transport Rate (mM/min)

Table 2 and 3: Activity 4: Simulating Filtration

Table #2: Solute Residue Presence in the Membrane Key: If solute residue was present on the membrane, it is indicated by a “+”. If solute residue was not present on the membrane, it is indicated by a “-”.

Membrane (MWCO)	Solute	20	50	100	200
	NaCl	+	+	+	+
	Urea	+	+	+	+
	Glucose	+	+	+	+
	Powdered Charcoal	+	+	+	+

Table 3: Filtration Rate and Amount of Solute Detected in Filtrate

Membrane (MWCO)	Solute	20	50	100	200
	Filtration Rate (ml/min)	1	2.0	5	10
	NaCl infiltrate (mg/ml)	0	4.81	4.81	4.81
	Urea in filtrate (mg/ml)	0	0	4.74	4.74
	Glucose in filtrate	0	0	0	4.9

	(mg/ml)				
	Powdered Charcoal (mg/ml)	0	0	0	0

Discussion

The first lab experiment, Simulating Dialysis (Simple Diffusion), demonstrated how only certain molecules pass through a selectively permeable membrane down its concentration gradient. The four membranes utilized in this experiment consisted of each one being different in pore size (MWCO). The smallest pore-sized membrane was 20 (MWCO), and the largest was 200 (MWCO). The solutes that were tested in this experiment were NaCl, Urea, Albumin, and Glucose.

The first solute tested, NaCl, showed that with a 20 (MWCO) membrane, no diffusion occurred into the right beaker. (Table 1) The NaCl molecules were evidently too large to pass through the 20 (MWCO) membrane because its pores were too small. Membranes 50, 100, and 200 (MWCO) did allow the NaCl to pass through. (Table 1) One of the reasons this occurred is because the pores in the above-mentioned membranes were large enough to permit the passage of the NaCl molecules. The other reason diffusion occurred is because the NaCl molecules moved down its concentration gradient and into the beaker filled with deionized water. For all three membranes, equilibrium was reached in ten minutes at an average diffusion rate of 0.0150 mM/min.

As for the solute Urea, the experiment conducted showed that no diffusion occurred with all four membranes. (Table 1) Urea should have passed through membranes 100 (MWCO) and 200 (MWCO) for the reasons that its molecules are small enough and Urea is also soluble. This experiment showed that none of the Albumin molecules diffused through any of the four membranes tested. (Table 1) This is because the Albumin molecules were too large to pass through the pores of all four membranes. The final solute tested in this experiment, Glucose, showed that the molecules only diffused through the 200 (MWCO) membrane. (Table 1) Equilibrium was reached in thirty-seven minutes at an average diffusion rate of 0.0040 mM/min.

The Glucose molecules were too large to diffuse through the 20 (MWCO), 50 (MWCO), and 100 (MWCO) membranes. The second experiment, Simulating Facilitated Diffusion, explained how carrier protein molecules in the membrane effectively transported molecules that are too large or are insoluble to diffuse through the membrane. The carrier proteins in this experiment were glucose carriers and the solution was a 2.00 (mM) and an 8.00 (mM) glucose concentration. The 2.00 (mM) glucose concentration was tested first with the 500 glucose carrier protein-membrane then the 700 and 900 glucose carrier protein membranes. The glucose transport rate for the membrane with 500 glucose carrier proteins was 0.0008 (mM/min). Graph 1) The membrane with 700 glucose carrier proteins showed a rate of 0.0010 (mM/min) and the 900 glucose carrier proteins membrane had a rate of 0.0012 (mM/min). (Graph 1) The 8.00 (mM) glucose concentration also showed an increase in glucose transport rate with membranes that contained more glucose carrier proteins. The membrane with 500 glucose

carrier proteins showed a rate of 0.0023 (mM/min). (Graph 1) Membranes that had 700 and 900 glucose carrier proteins showed a rate of 0.0031 and 0.0038 (mM/min). (Graph 1) These results show that with an increase in the amount of glucose carrier proteins in the membranes, transport of the glucose molecules in the concentration is more effective.

A higher concentration of glucose (8.00 mM) also increases the rate of glucose transport in a membrane with the same amount of glucose carrier proteins as a lower glucose concentration (2.00). In the final experiment, Simulating Filtration, four different solutes were forced through four membranes that contained separate pore sizes by the use of hydrostatic pressure. After each experimental run was conducted, the membrane analyses showed that residue from all four solutes was detected on each membrane. (Table 2) This indicates that some solutes did not filter through the membrane. The filtration rate (ml/min) increased as membranes with larger pores were utilized.

This happened because the solute molecules were able to transport through a particular membrane at a faster rate being that the membranes' pores were larger. The filtrate in the bottom beaker was analyzed and no solutes were detected with the 20 (MWCO) membrane. (Table 3) With the 50 (MWCO) membrane, only NaCl was detected in the filtrate at 4.81 (mg/ml). (Table 3) The 100 (MWCO) membrane showed to have NaCl at 4.81 (mg/ml) and Urea at 4.74 (mg/ml) present in the filtrate. (Table 3) Glucose and powdered charcoal were not present. The last membrane with pore size 200 (MWCO), had the solutes NaCl at 4.81 (mg/ml), Urea at 4.74 (mg/ml), and

Glucose at 4.39 (mg/ml) detected in the filtrate. (Table 3) Powdered charcoal was not detected in this filtrate. Table 3) The molecules in powdered charcoal were too large to pass through any of the membranes tested. The 20 (MWCO) membrane pores were too small to allow any solute molecules to pass through. The membranes that contained larger pores allowed the solutes with larger pores to pass through. The amounts (mg/ml) of the same solute detected in the filtrate were the same for each membrane. (Table 3) This is because the pressure that was released into the top beaker remained at 50 (mmHg) for all experiment runs.

References:

1. Marieb, Elaine N., Mitchell, Susan J. (2008). Exercise 5B. Human Anatomy & Physiology Laboratory Manual Ninth Edition (pp. PEx-5 – PEx-13). San Francisco, California: Pearson Benjamin Cummings.